# Retroviruses: Key to discovery of oncogenic microRNAs

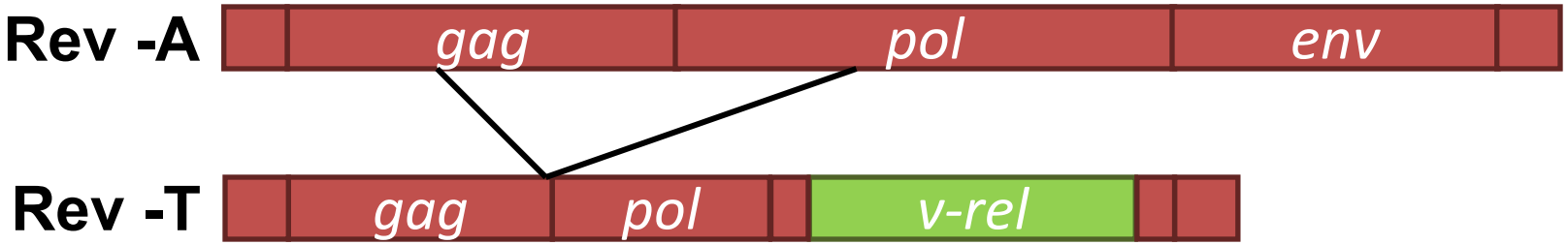
Karen Beemon
Johns Hopkins University
Baltimore, Maryland, USA

# Avian Retroviruses

- Avian leukosis virus


- Rous sarcoma virus (Replication competent)


- Reticuloendotheliosis virus strain T (Replication deficient)



# Why study cancer in chickens?

1. First cancer inducing viruses : Rous sarcoma virus (Rous, 1910)
   Avian leukosis virus (Ellerman and Bang, 1908)

2. First oncogene identified: src (Duesberg, 1975)

3. First tyrosine protein kinase: src (Hunter, 1980)

4. First retroviral insertional mutagenesis: myc (Hayward,1981)

5. First oncomiR activated in tumors: bic = miR-155 (Hayward, 1989)

6. TERT first activated by insertional mutagenesis (Beemon, 2007)

7. Are other types of non-coding RNAS important for cancer?

# ONE HUNDRED YEARS OF RETROVIRUSES

Peyton ROUS

AB, 1900; MD 1905, Johns Hopkins University

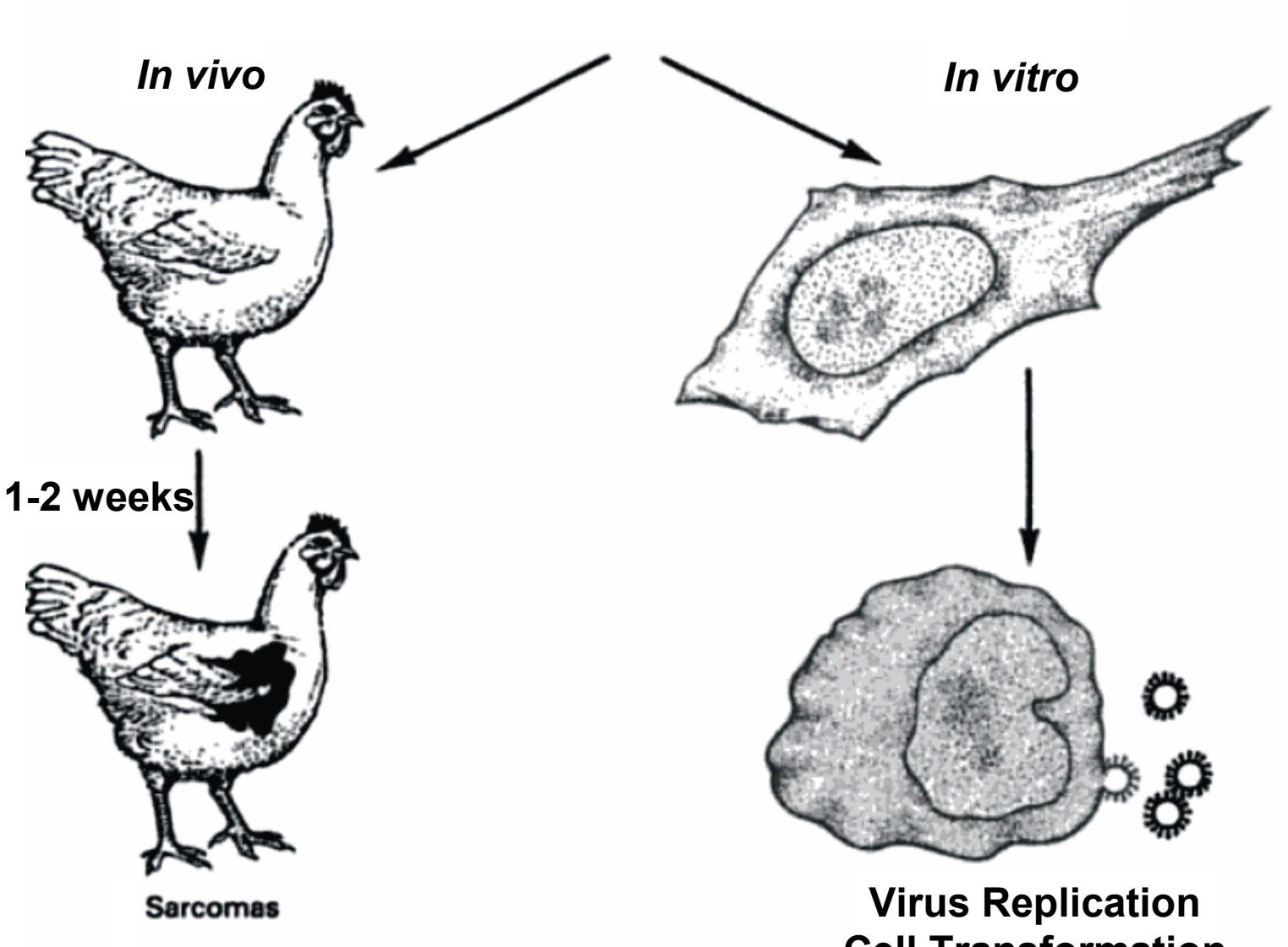
Rous sarcoma virus 1910, Rockefeller Institute

Nobel Prize 1966

# Peyton Rous
# AB 1900, MD 1905
# Johns Hopkins University

*Age twenty Johns Hopkins University*

# Rous sarcoma virus oncogenesis

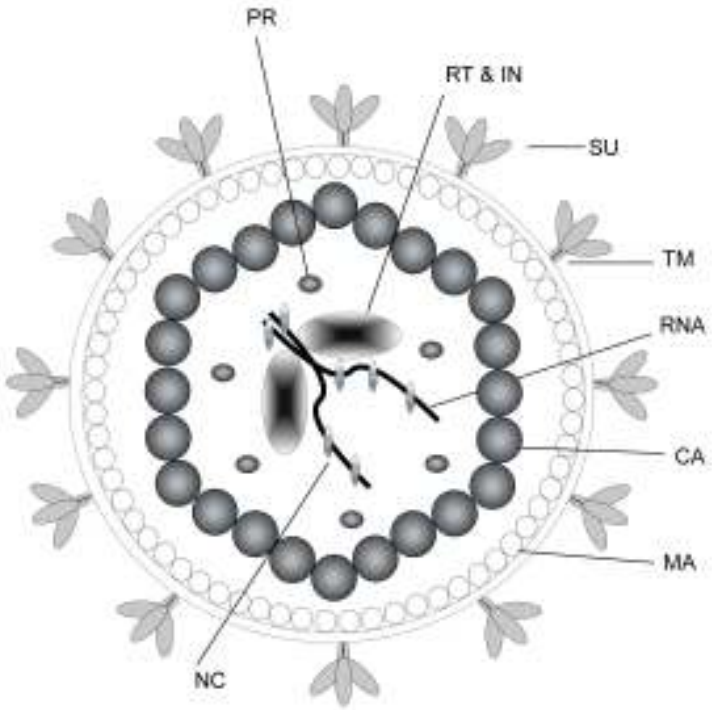


# Avian Retroviruses

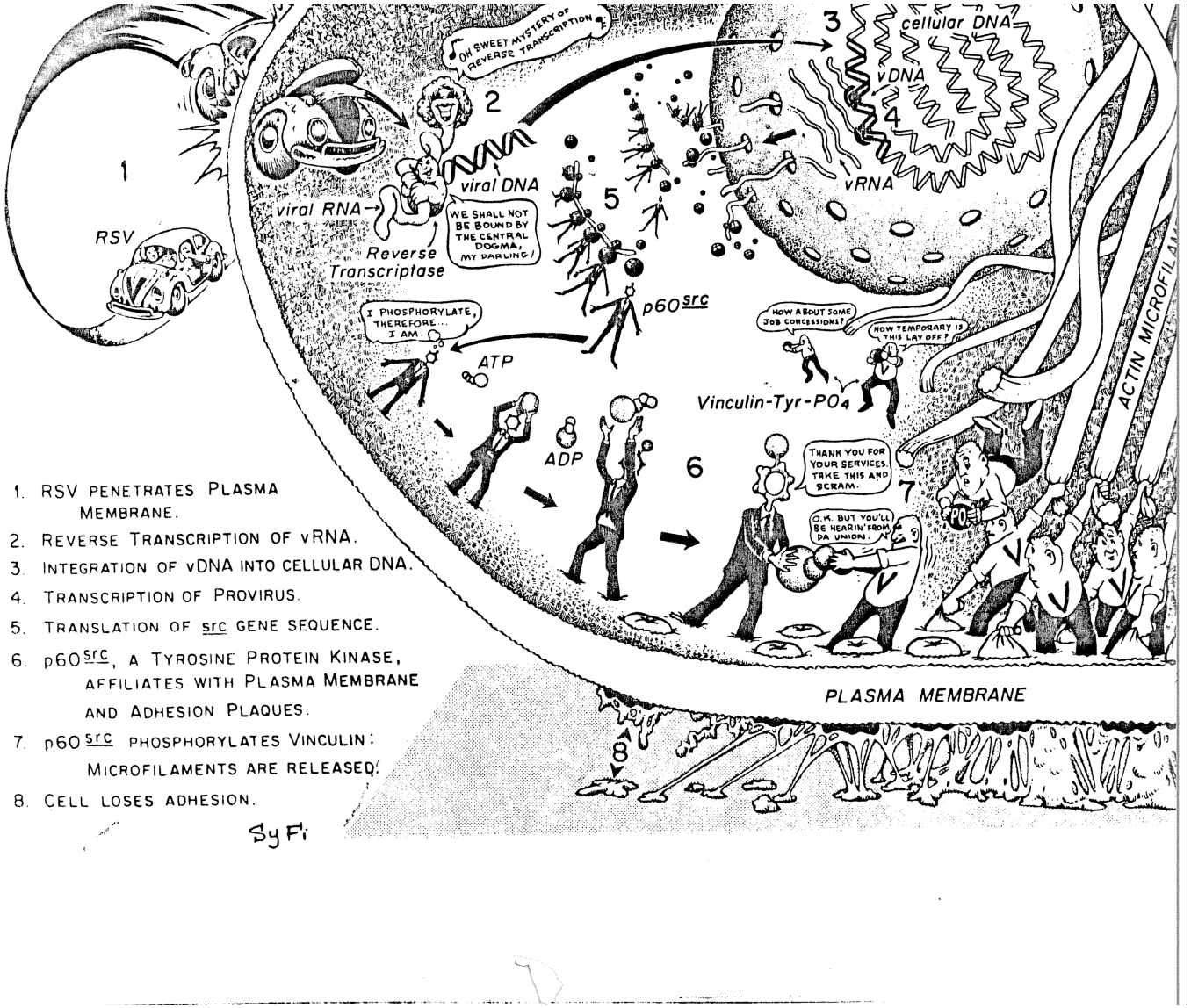
Rous sarcoma virus (RSV) (replication competent)


Captured oncogene

Wang et al. 1975

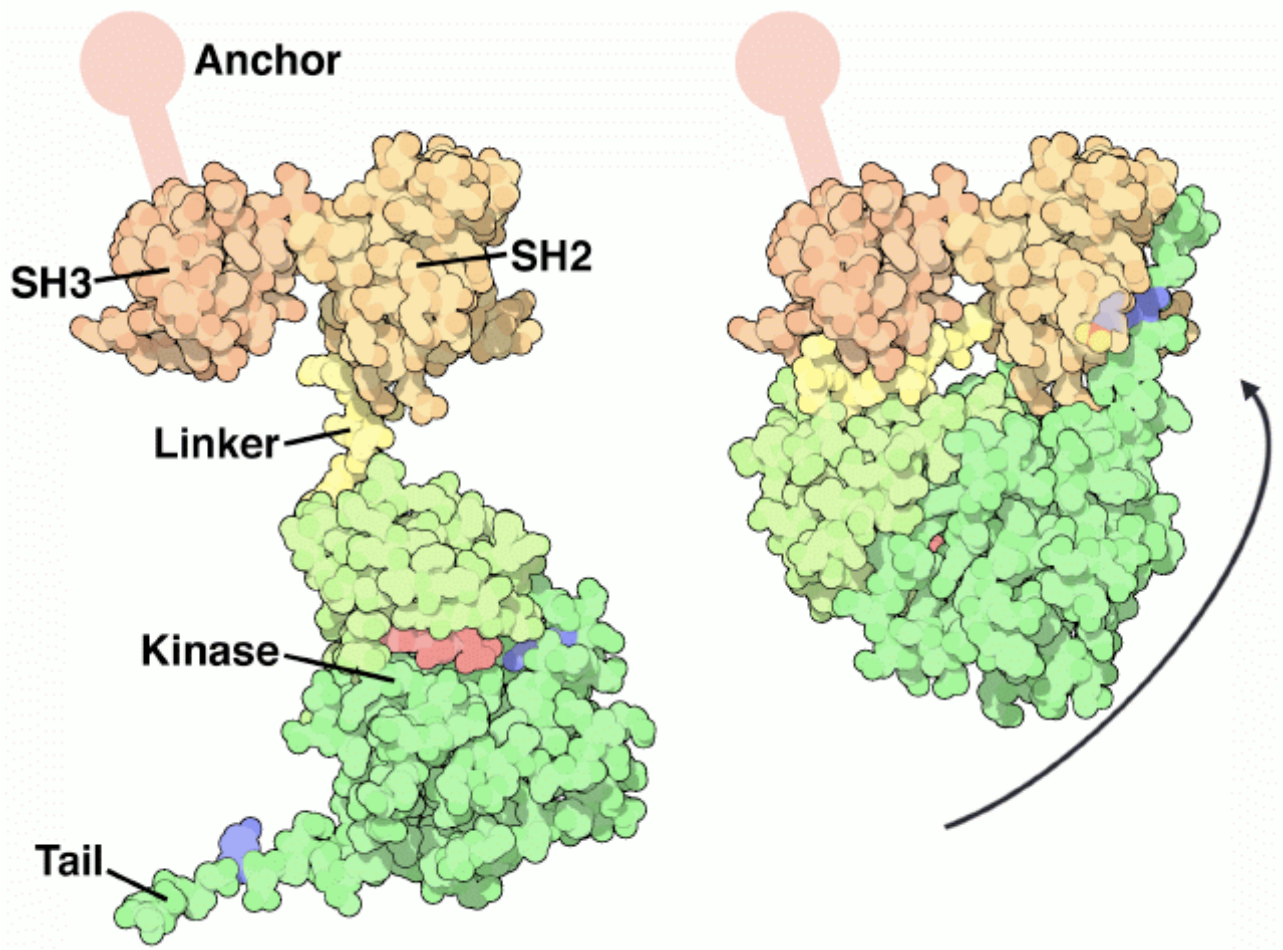


# p60$^{Src}$ is a tyrosine kinase




Jamie Simon

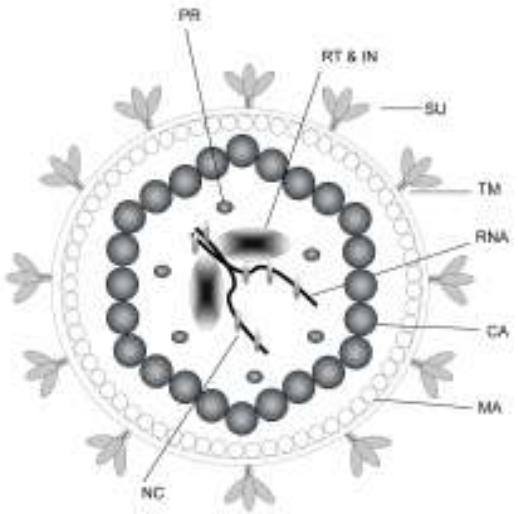
# c-Src inactivation

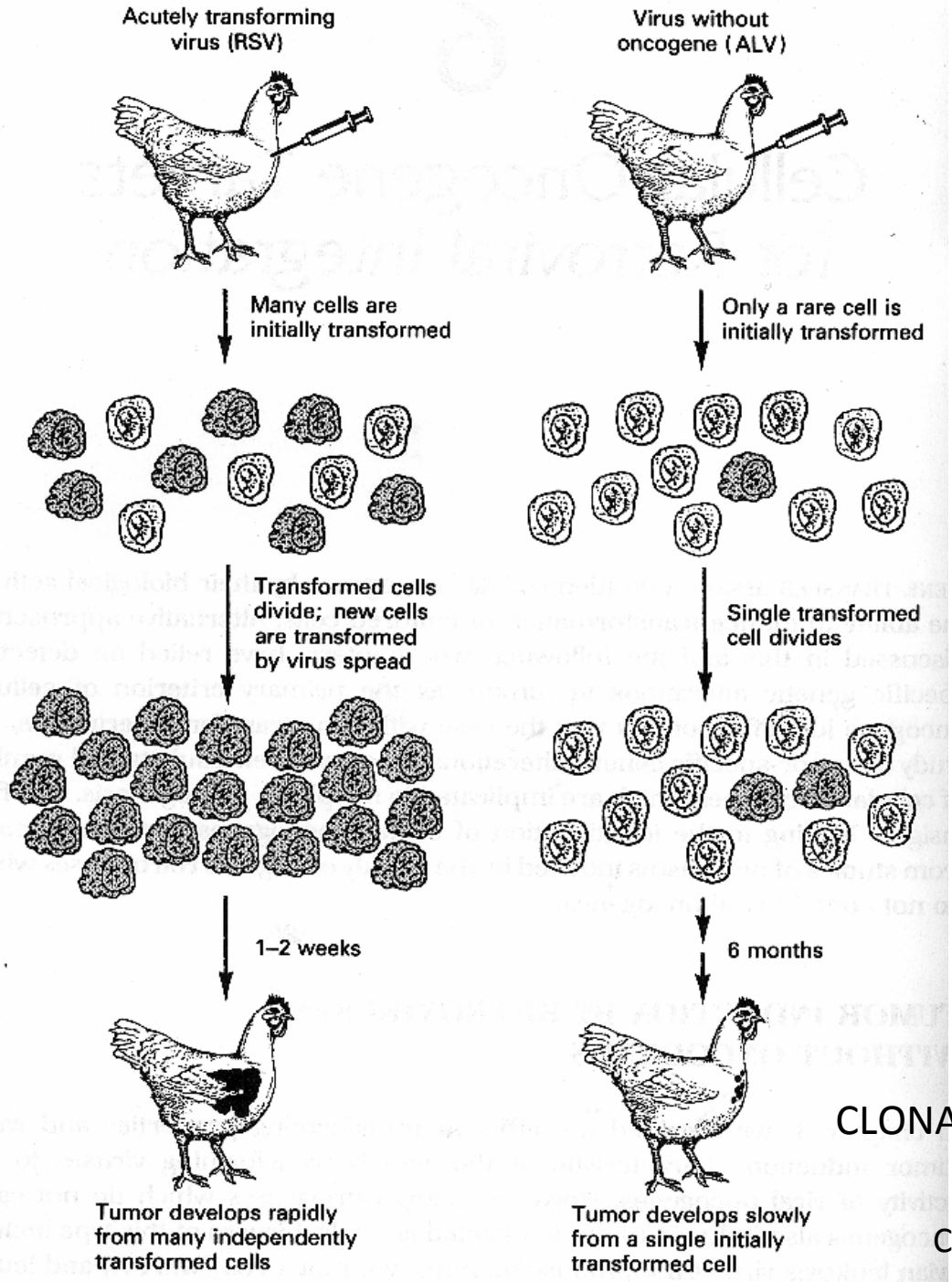


# How does ALV cause lymphomas?

Avian leukosis virus (ALV)


Rous sarcoma virus (RSV)

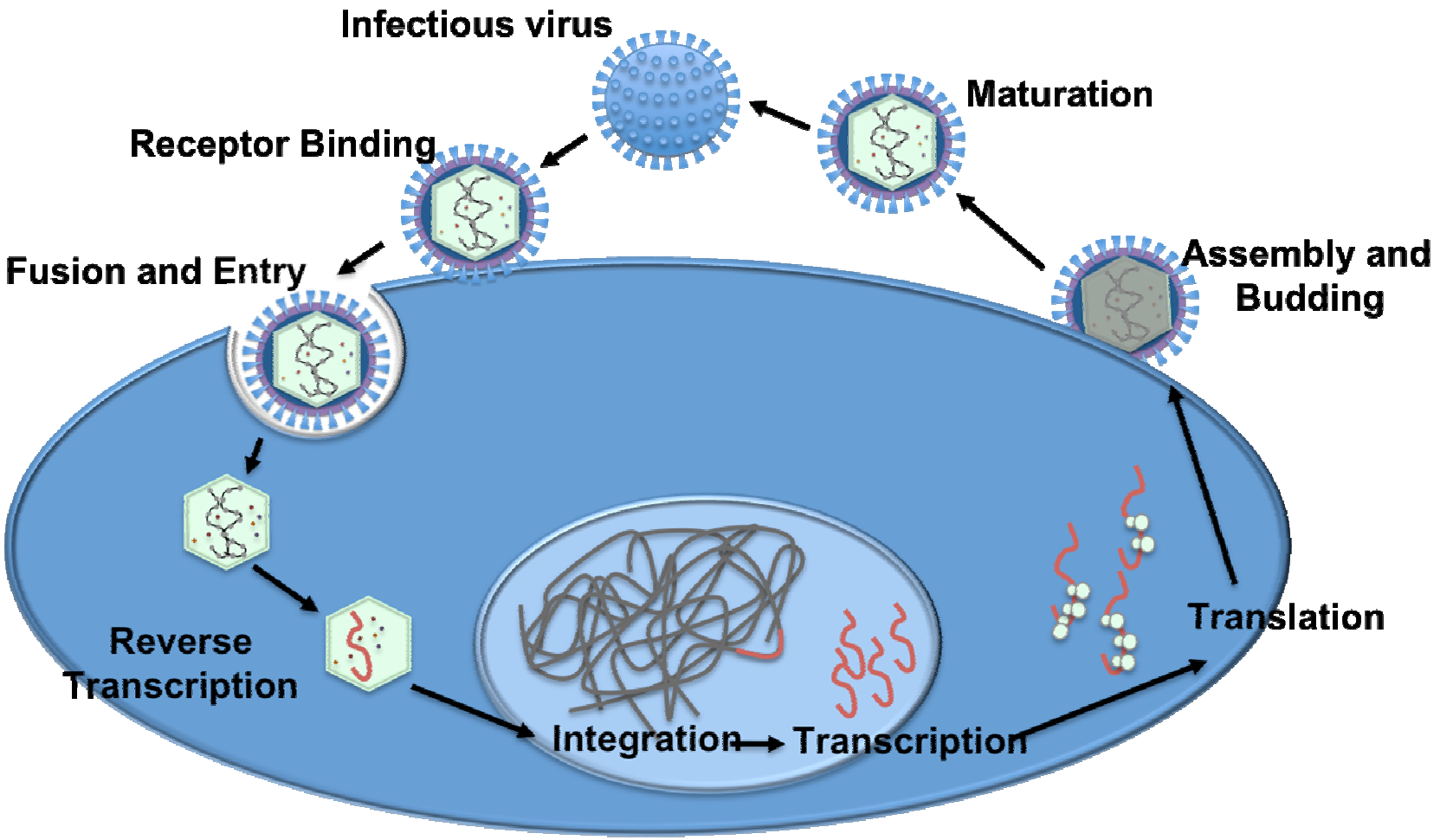





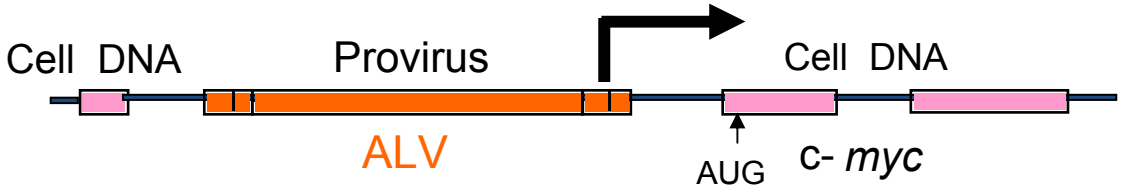

CLONAL TUMORS

Cooper, Oncogenes

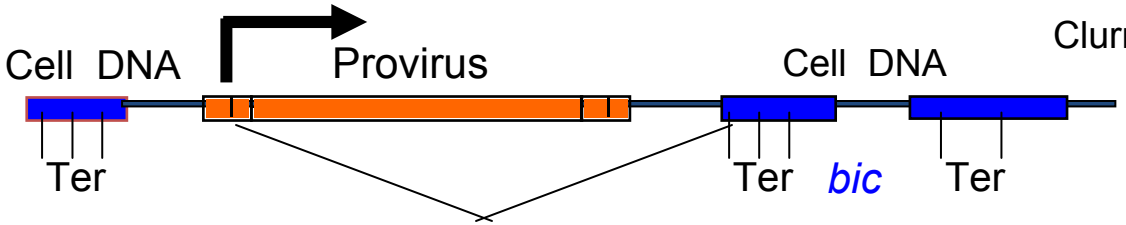
# Retroviruses integrate proviral genomes into host DNA



# ALV integrates in B-cell integration cluster (bic) and myc genes in lymphomas




Hayward et al. 1981




Clurman & Hayward 1989

MOLECULAR AND CELLULAR BIOLOGY, June 1989, p. 2657–2664
0270-7306/89/062657-08$02.00/0

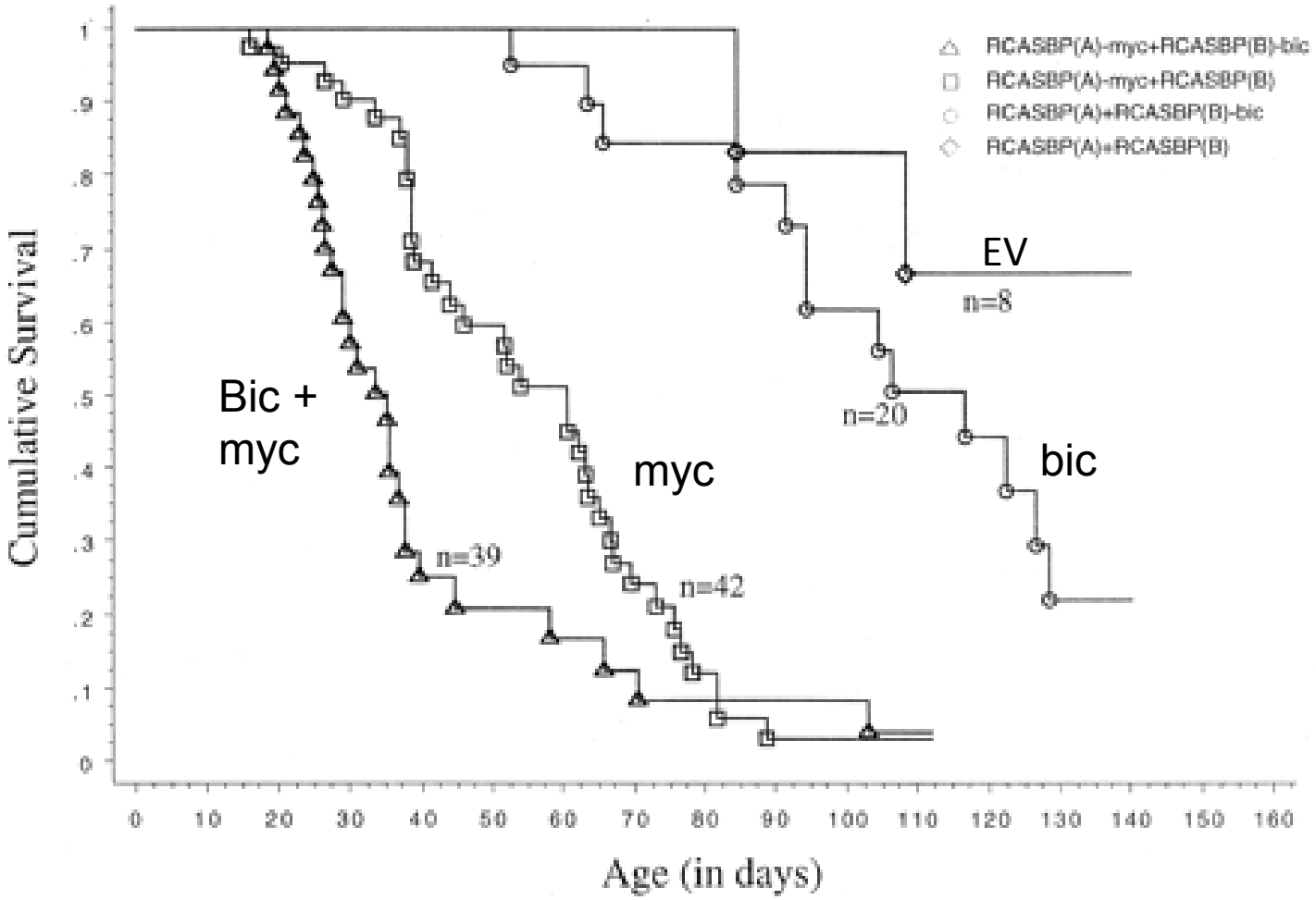
Vol. 9, No. 6

## Multiple Proto-Oncogene Activations in Avian Leukosis Virus-Induced Lymphomas: Evidence for Stage-Specific Events

BRUCE E. CLURMAN† AND WILLIAM S. HAYWARD*
*Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021*

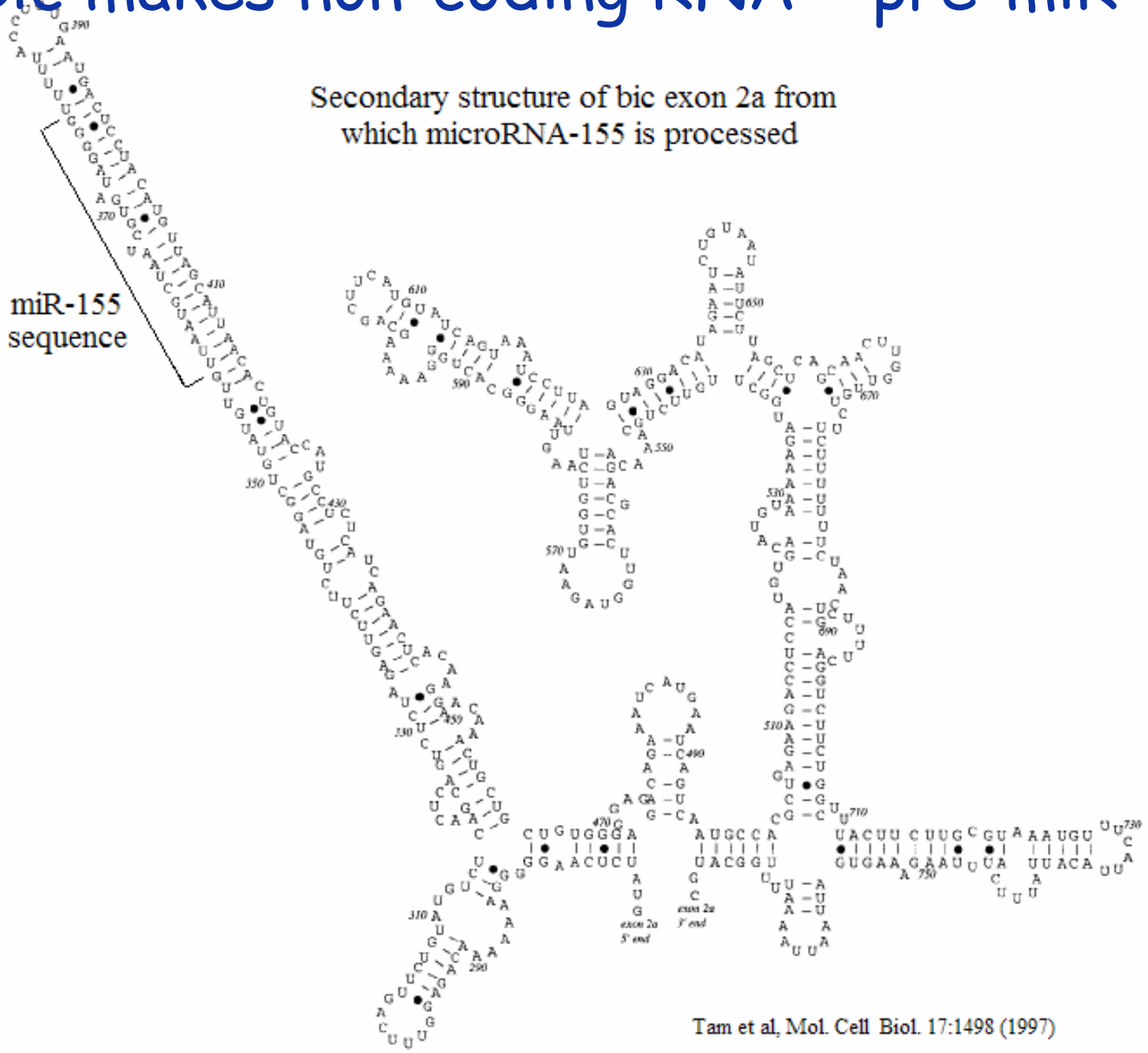
Received 3 February 1989/Accepted 20 March 1989

# *bic* and *myc* oncogenes cooperate in tumorigenesis in chickens

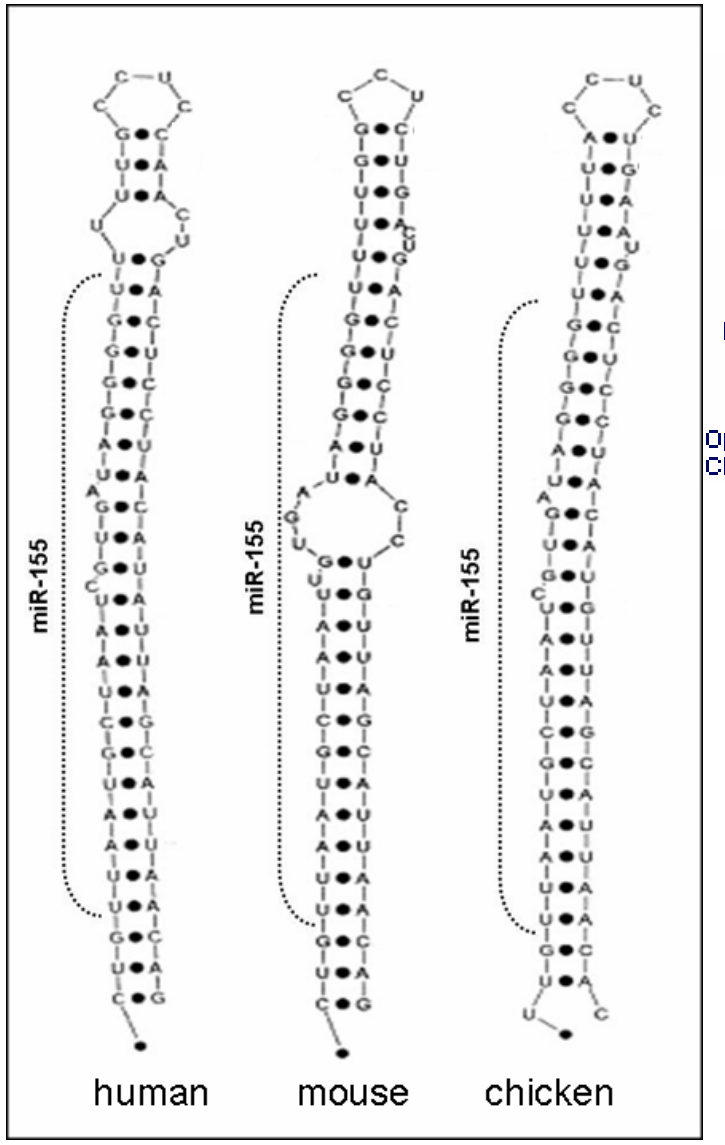


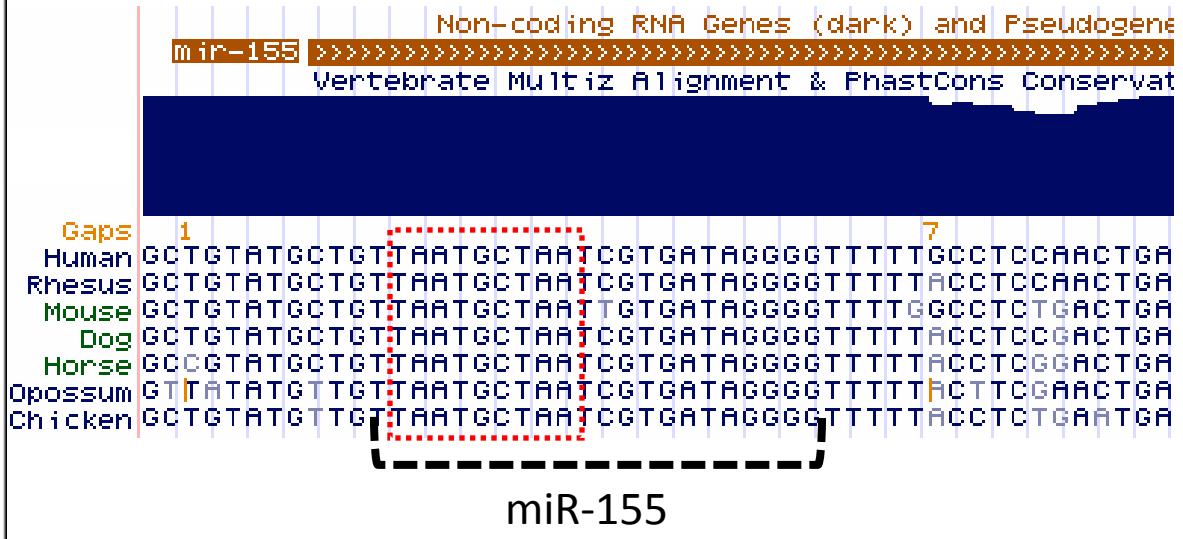

Tam et al. (2002) JVI

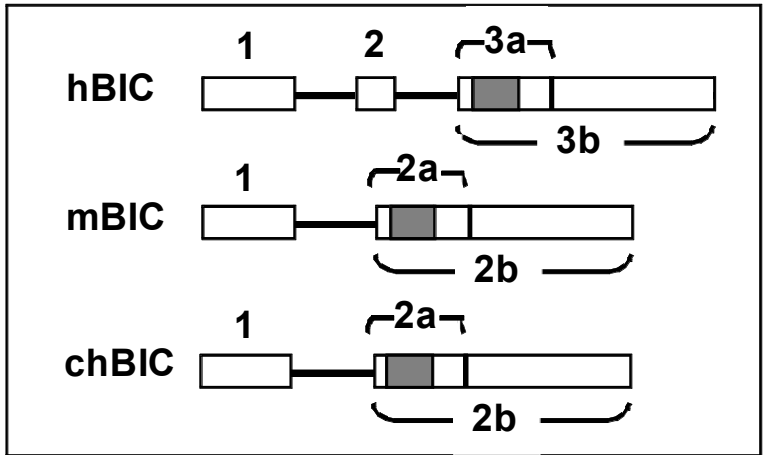
# Bic makes non-coding RNA = pre-miR-155



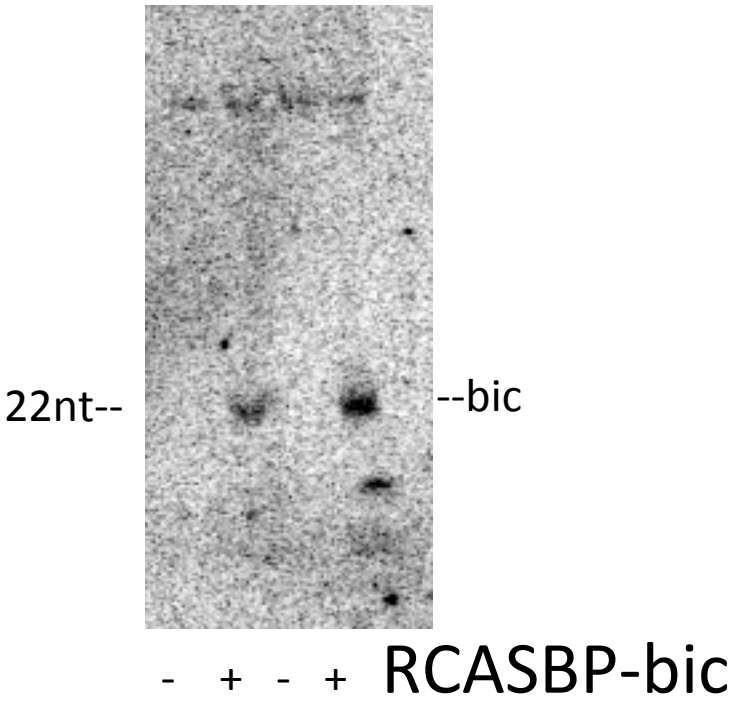
# miR-155 is conserved across species



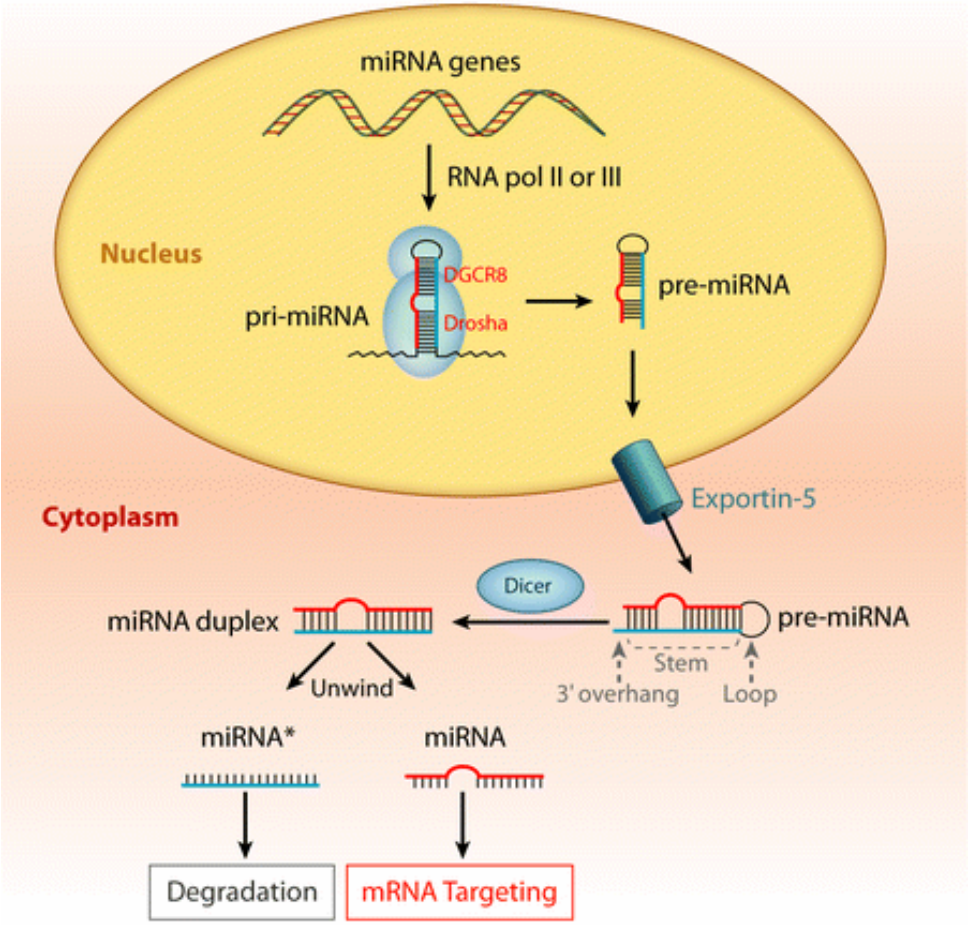



# *bic* processed to 22 nt miR-155: the first oncomiR

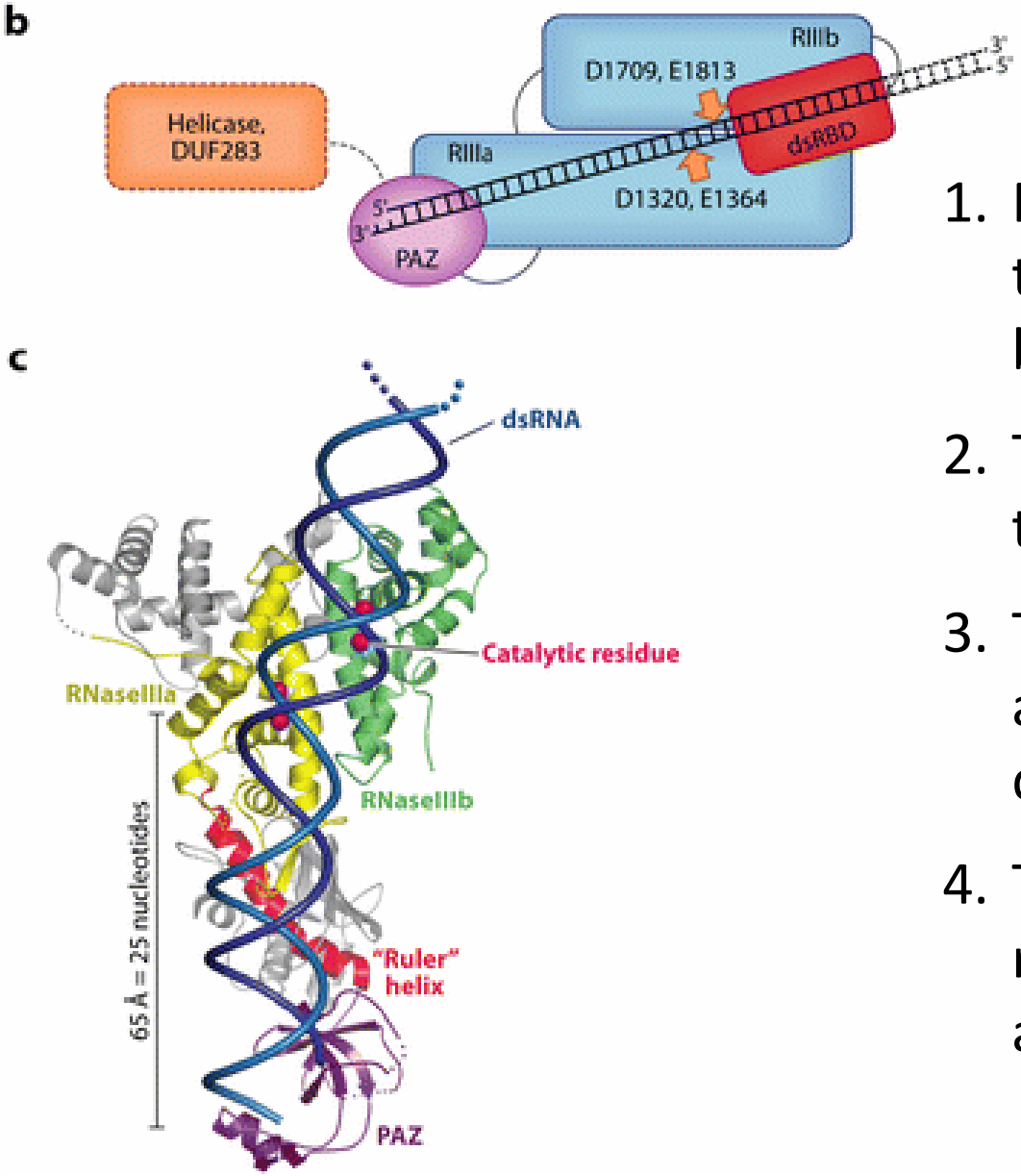


# Biogenesis of miRNAs



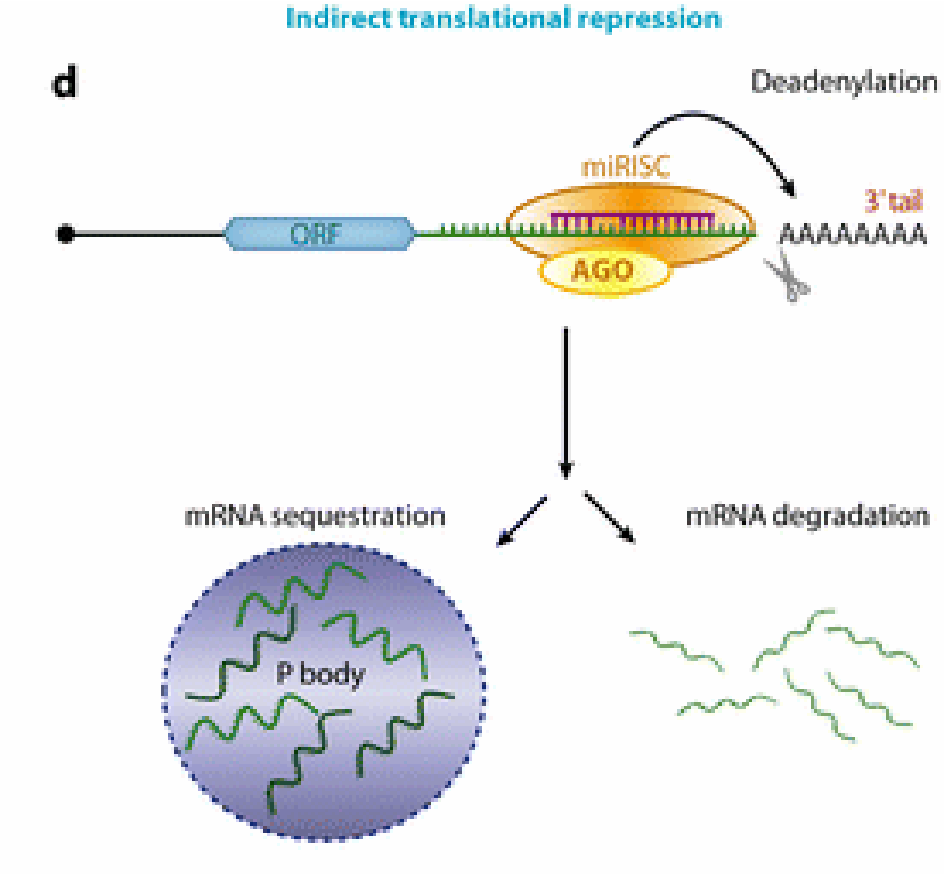

1. Transcribed like protein coding genes – RNA pol II
2. Hairpin is cleaved from pri-miRNA by Drosha
3. Pre-miRNA is transported by Exportin 5
4. Pre-miRNA is cleaved by Dicer
5. miRNA is loaded into RISC

# Dicer – an RNASEIII endonuclease



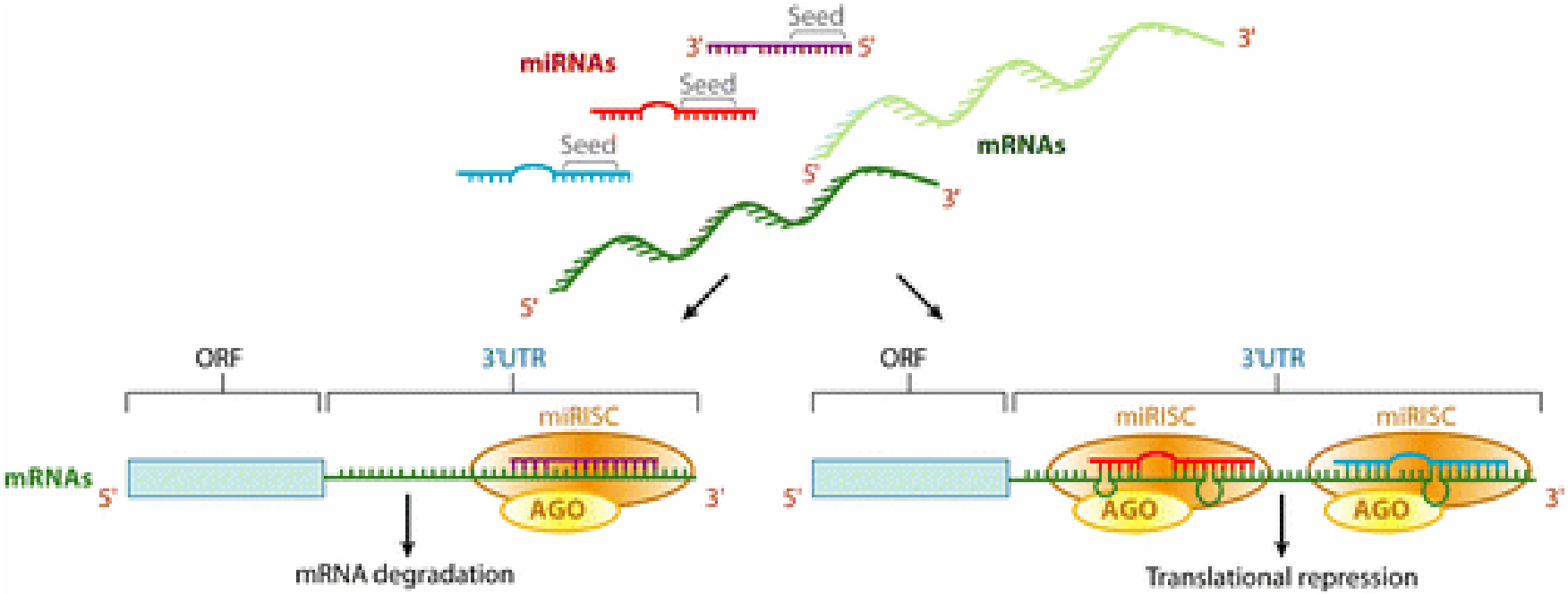

1. PAZ domain of Dicer binds the 3' 2nt overhang created by Drosha cleavage
2. The dsRBD of Dicer positions the 70 nt hairpin
3. The catalytic residues are about 25 nts from the PAZ domain
4. This distance acts as ruler resulting in miRNAs that are about 21 – 25nt in length

# mRNA destabilization and degradation by miRNAs




1. mRNA degradation is the predominant way that miRNAs inhibit protein synthesis
2. miRISC can destabilize mRNAs by promoting deadenylation or decapping
3. miRISC sequester mRNAs to P-Bodies for storage and eventual degradation

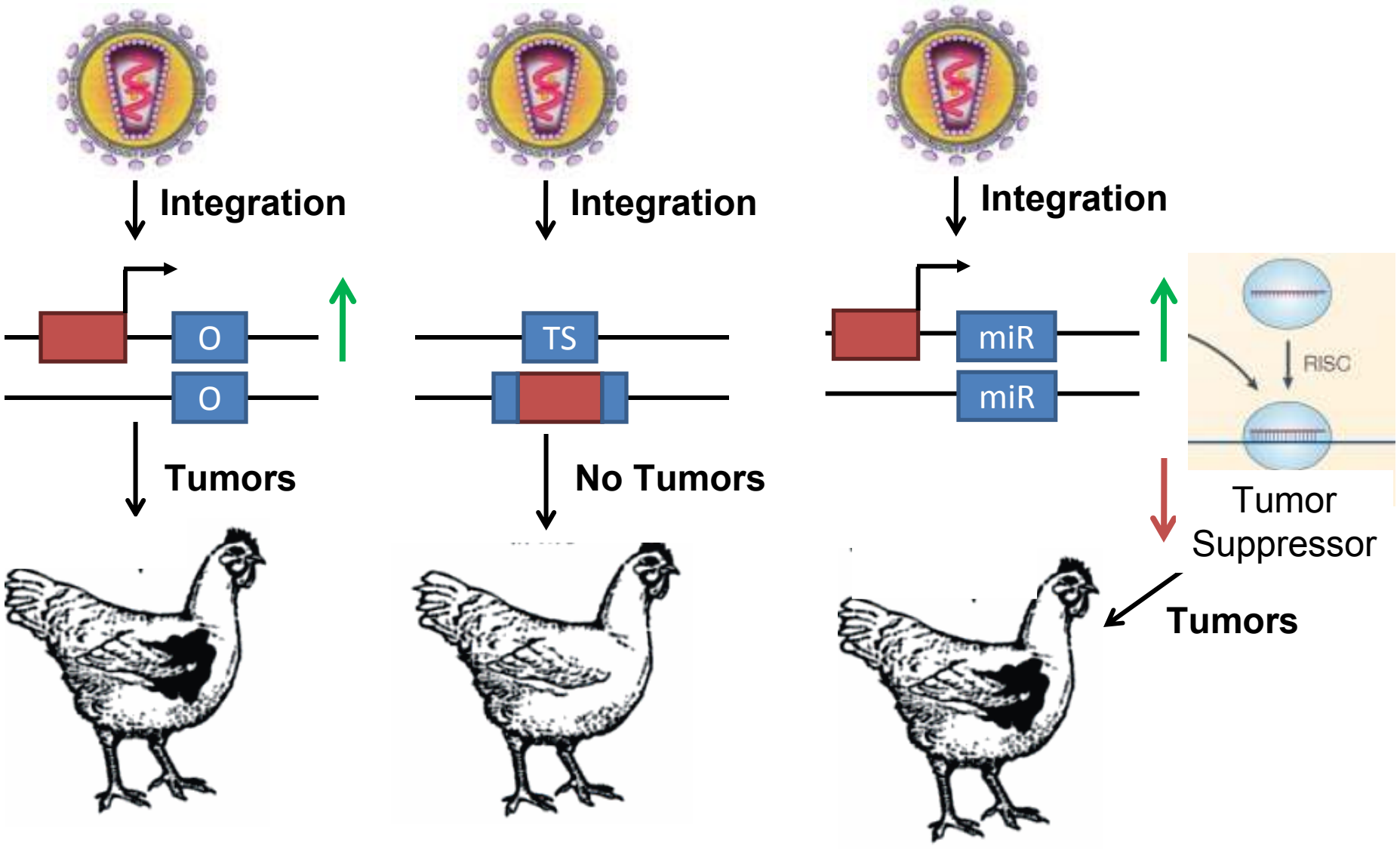
# Target selection by miRNAs




1. Seed sequence (nt 2 – 8) is important for target mRNA identification
2. miRNAs usually bind the 3'UTR of mRNAs
3. miRNA binding site usually more than 20 nt from the stop codon
4. miRNA binding site usually in AU – rich regions

- What are the targets of miR155?

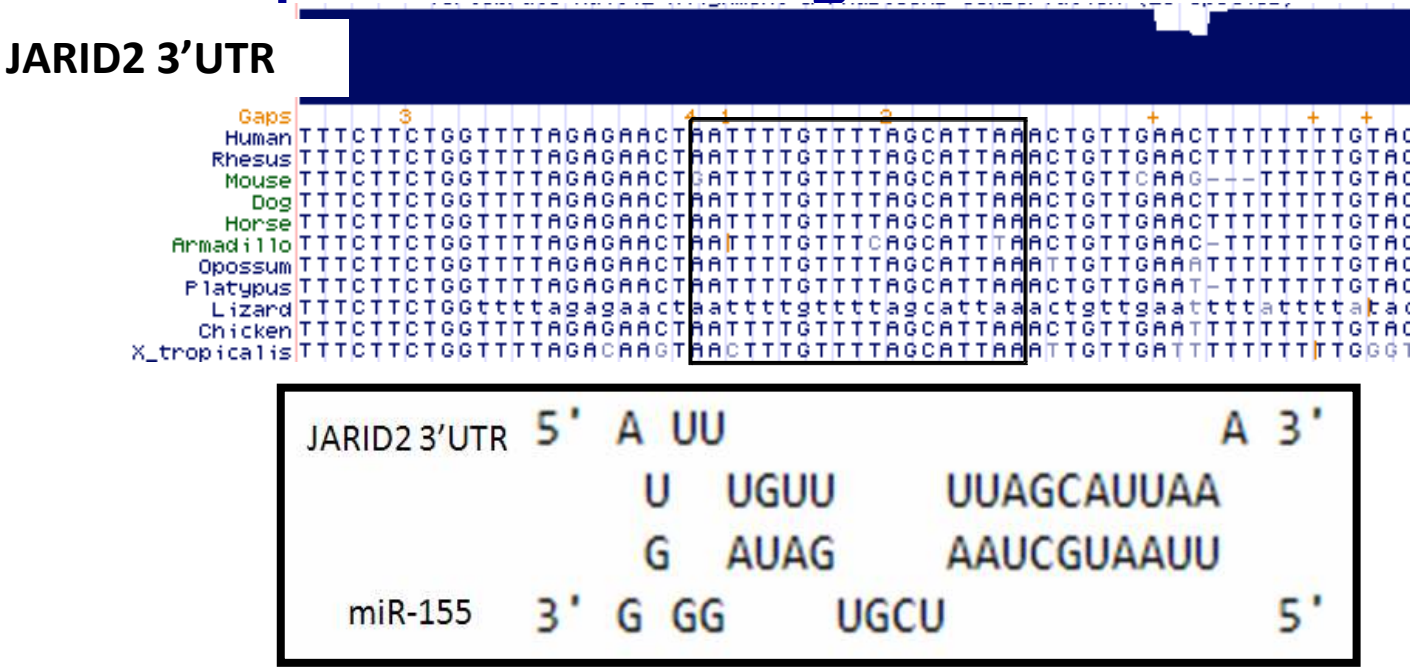
# miRNAs upregulated by retroviral insertions may target tumor suppressors



# Microarray of RCAS(*bic*)-infected CEFs

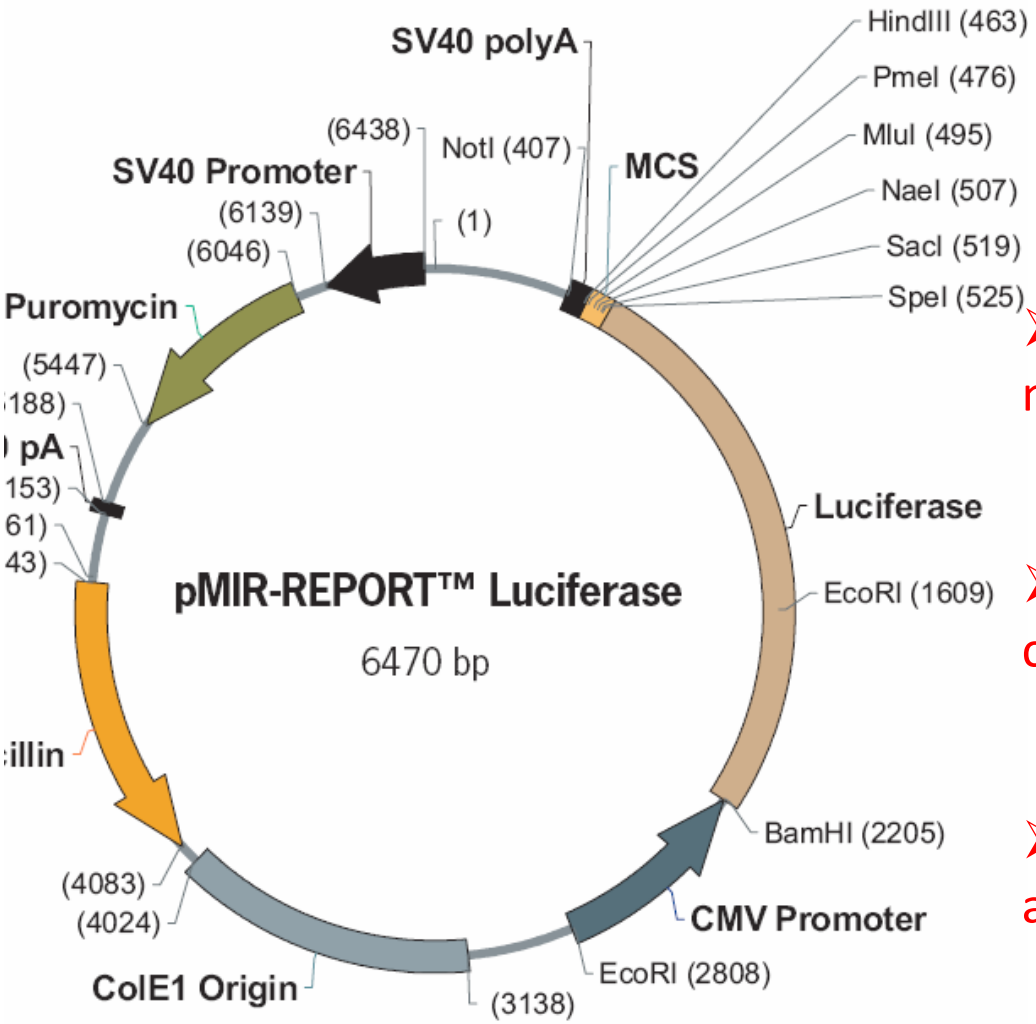
| Gene name | Fold down |
|---|---|
| **Sorting Nexin 12** | 3.71 |
| **JARID-2 (Jumonji)** | 2.50 |
| Matrix Gla-protein precursor (MGP) | 4.14 |
| similar to secreted protein Isthmin | 4.27 |
| Bos taurus similar to downregulated in ovarian cancer 1 isoform 2 | 4.52 |
| similar to tumor necrosis factor related protein 4 | 4.91 |
| similar to deleted in colorectal carcinoma | 5.29 |
| Semaphorin 3C precursor (Collapsin-3) (COLL-3) | 5.49 |
| similar to PXMP4 OR peroxisomal membrane protein 4 | 6.02 |
| collectin sub-family member 12 (COLEC12) | 6.30 |
| Serinus canaria growth-associated polypeptide (GAP-43) | 6.95 |
| Pgo2 mRNA for Primglo2 | 7.97 |

# Jumonji / JARID2 is most conserved predicted target of miR-155



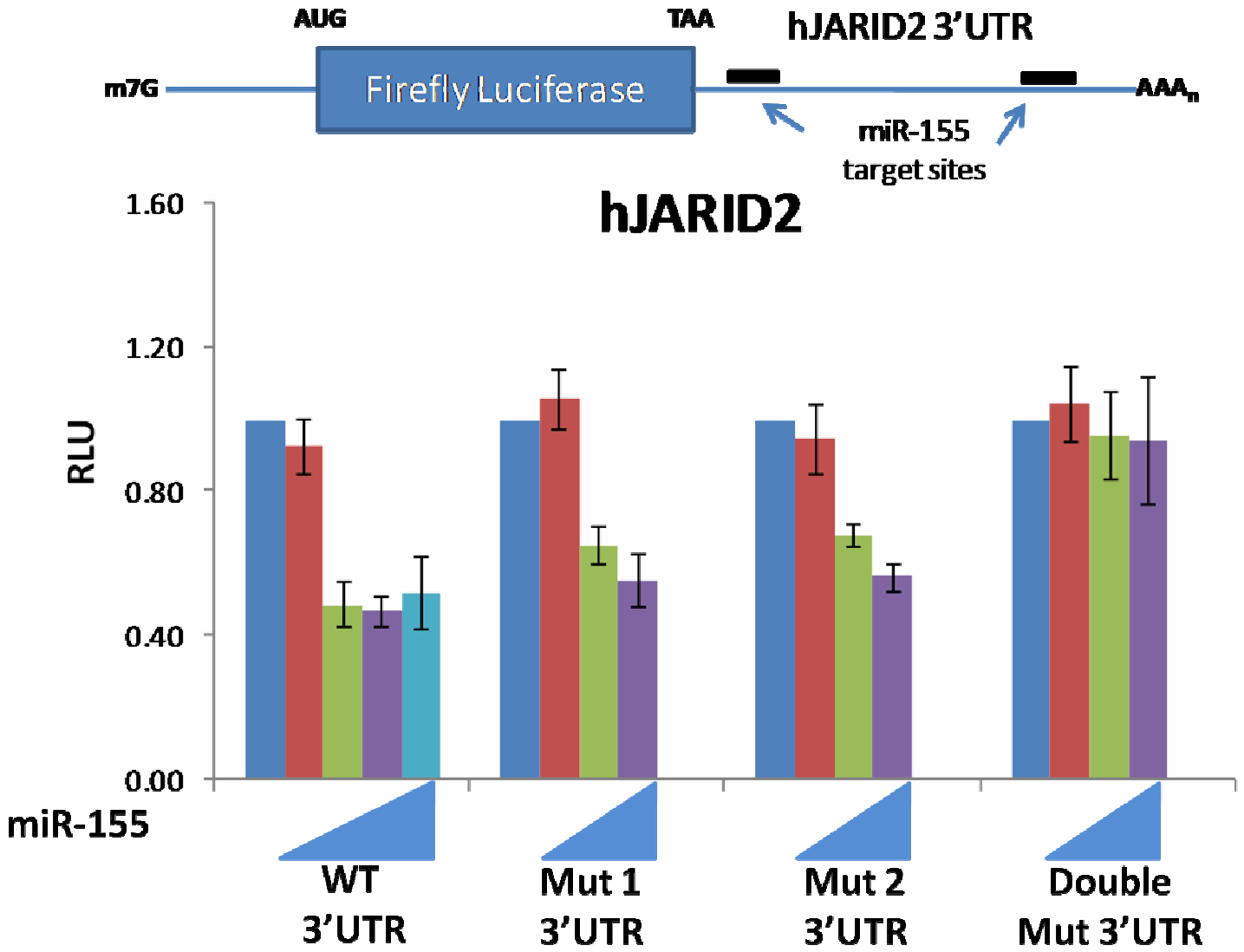

- potentiates Retinoblastoma gene
- Over-expression decreases cell growth
- recruits PRC2 (H3K27m2/3), essential for differentiation of ES cells

# Assay potential target 3'UTRs in luciferase reporter




- Identify potential targets from microarray, miRBase, miRanda, etc.
- PCR amplify the 3'UTR of targets and clone into pMIR-Report.
- Transfect cells and assay for luciferase activity.

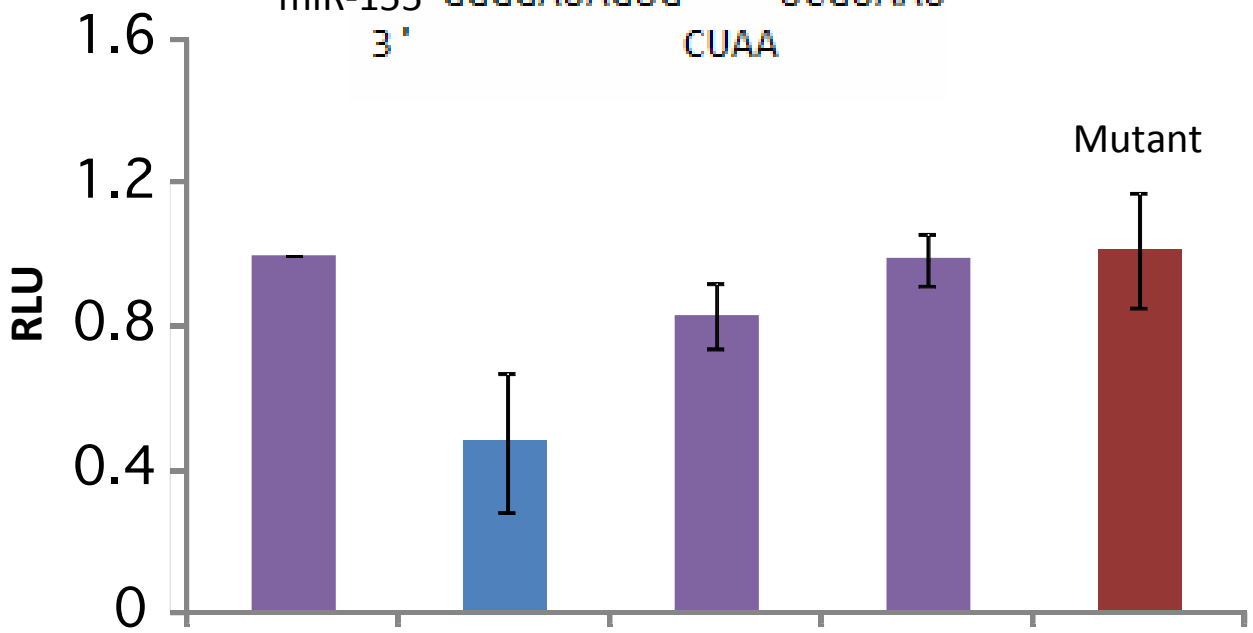
# hJARID2 is a target of miR-155




Bolisetty et al., J. Virol. 2009

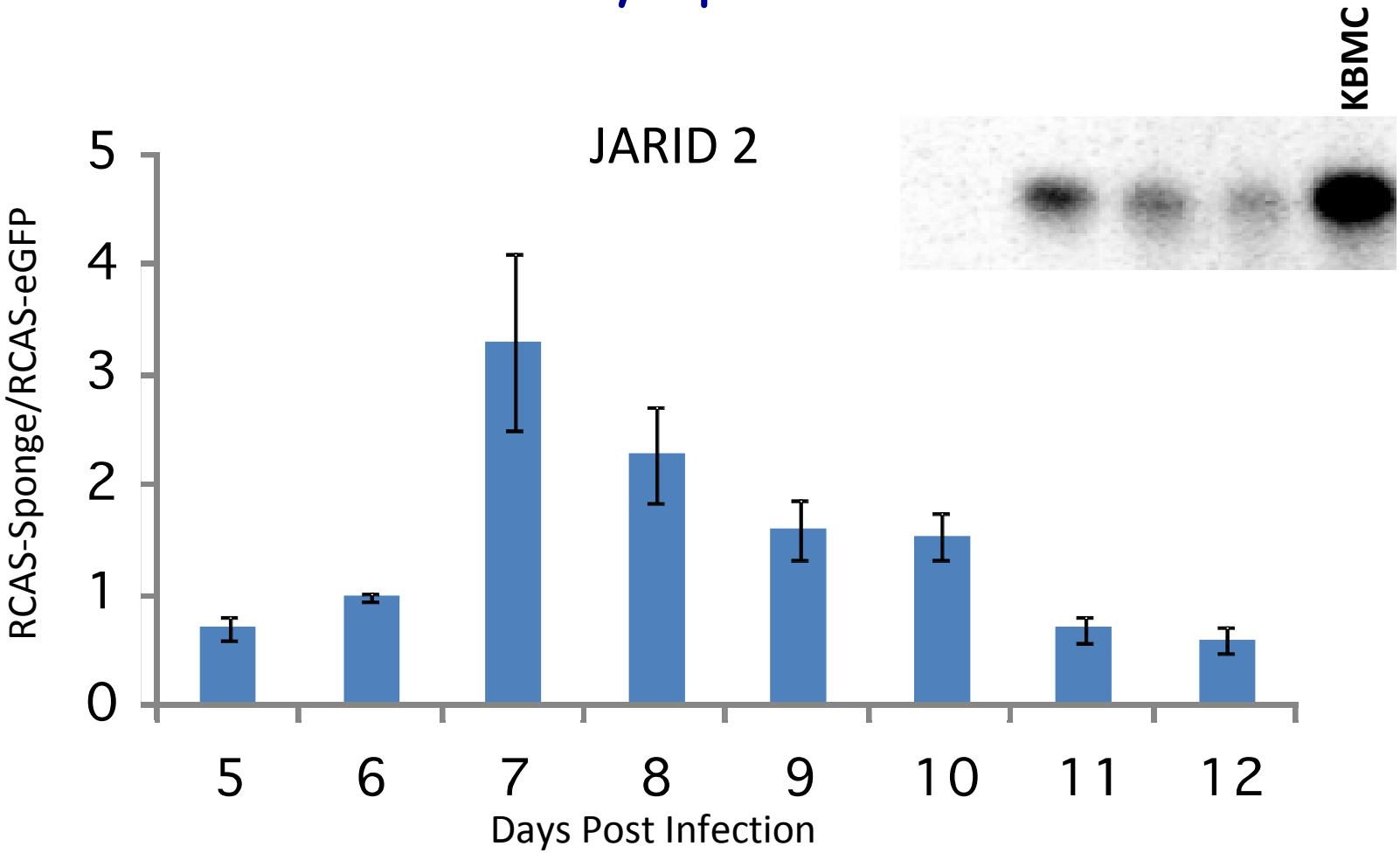
# miR-155 sponge abrogates repression of endogenous JARID2


```
        5'              CUAA
Sponge  CCCCUAUCAC      AGCAUUA
miR-155 GGGGAUAGUG      UCGUAAU
        3'              CUAA
```



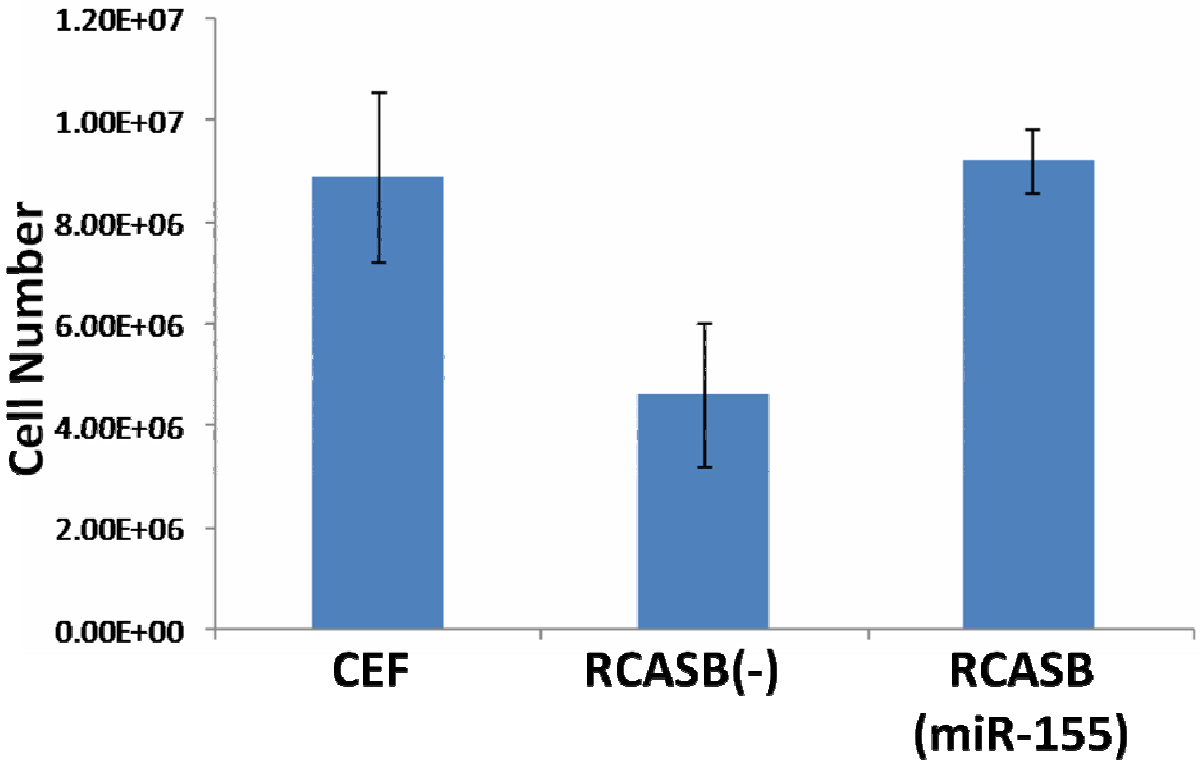

| | | | | | |
|---|---|---|---|---|---|
| Luc –JARID2– 3'UTR | + | + | + | + | – |
| Luc –JARID2-Mutant– 3'UTR | – | – | – | – | + |
| pcDNA3.1 – miR-155 | – | + | + | + | + |
| MS2 - Sponge | – | – | + | ++ | – |

# JARID2 is an endogenous target of miR-155 in B-Cell Lymphomas




$$\text{Fold Change} = \frac{\text{JARID2 mRNA levels in Sponge infections}}{\text{JARID2 mRNA levels in eGFP infections}}$$

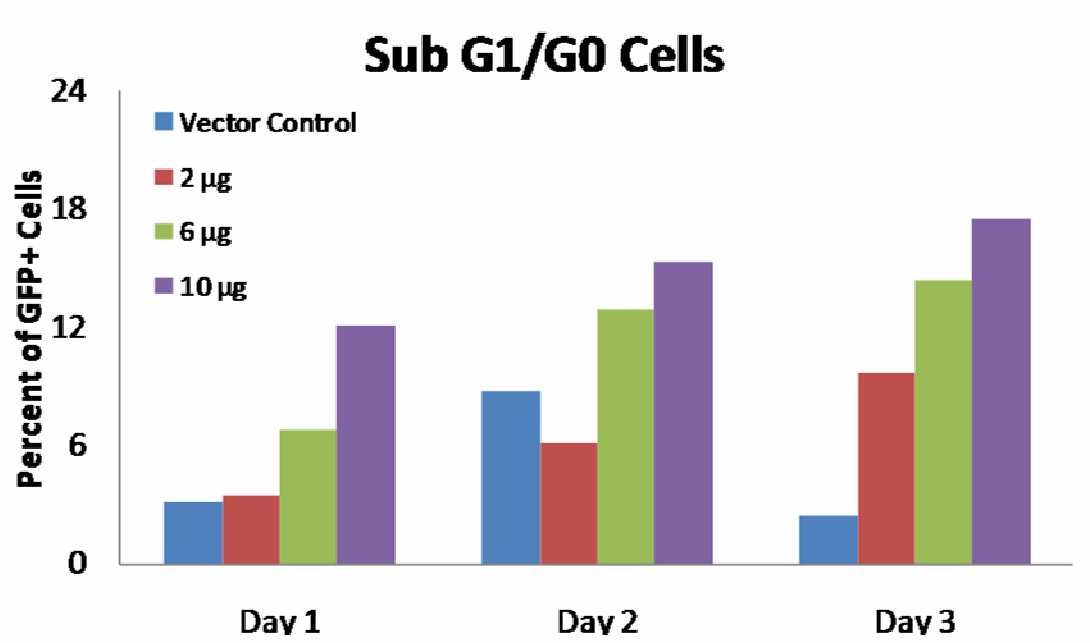
# miR-155 promotes cell survival

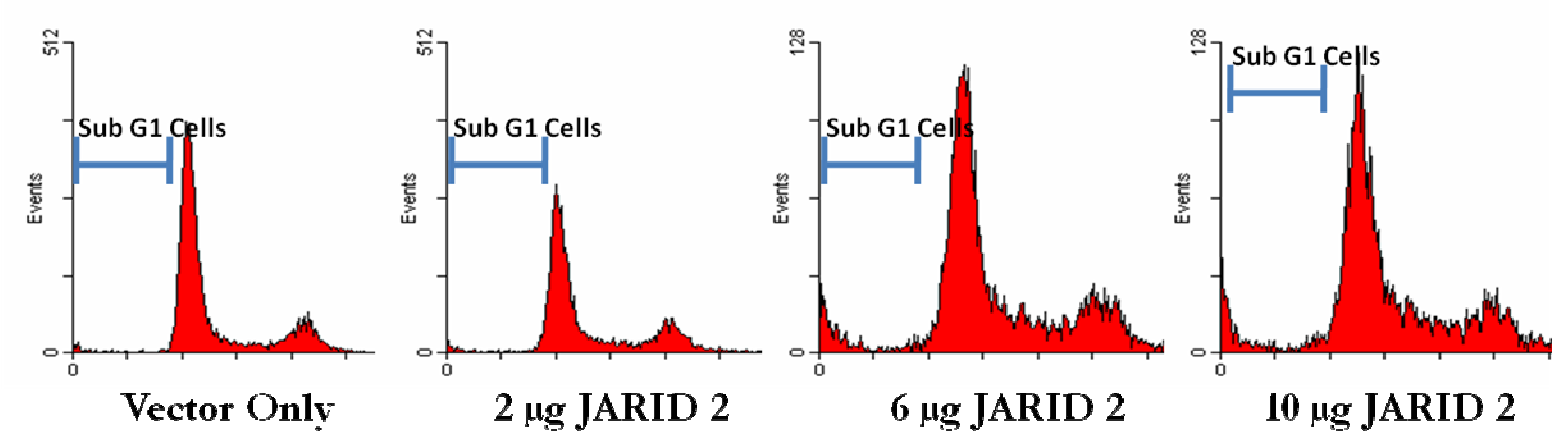



What targets of miR-155 are involved?

Mohan Bolisetty

# Over-expression of JARID2 increases apoptosis



# Summary

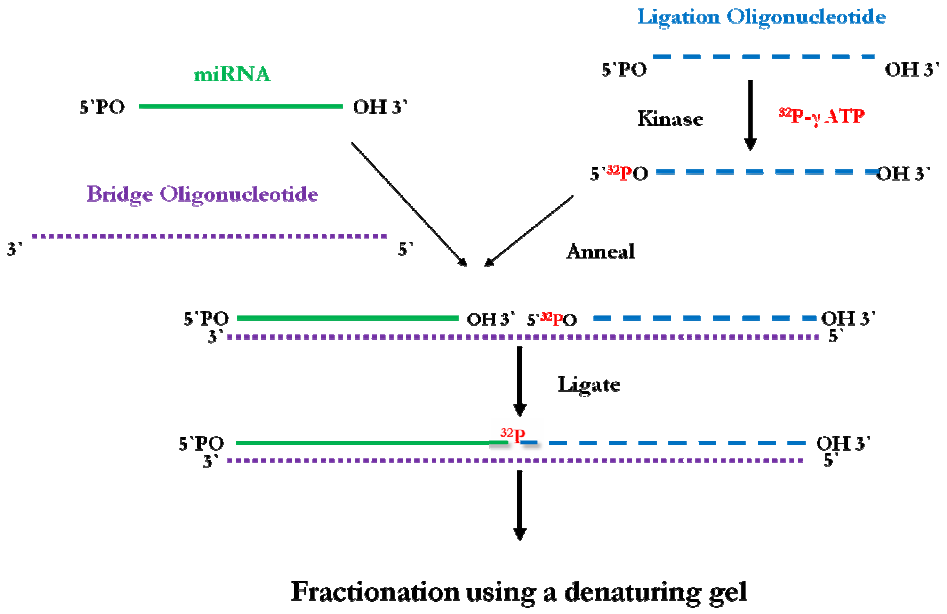
1. miR-155 (*bic*) is first oncomiR (ALV induced tumors)
2. Multiple targets validated including Jumonji /JARID-2
3. miR-155 is Anti-apoptotic (Cooperates with *myc*)
4. Bic (mir155) has important roles in oncogenesis, inflammation, immune function, cardiac function, & KSHV
5. Cancer biomarker - advanced human B cell lymphomas

# Bic important for cancer, heart, inflammation and the immune system

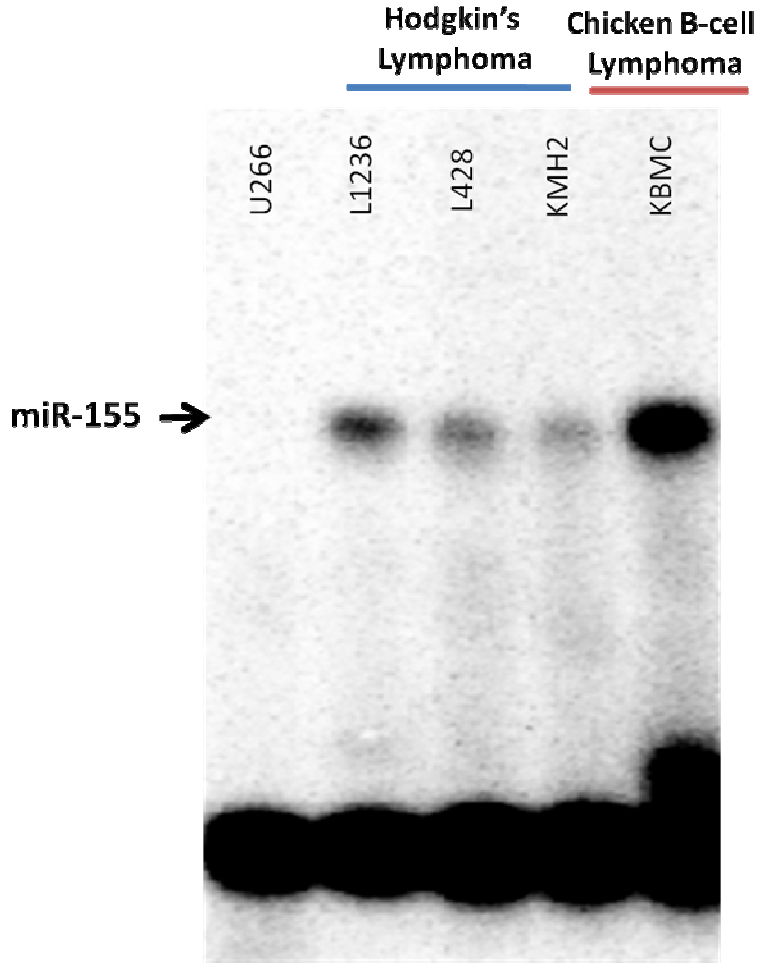
- miR-155 up-regulated in Hu B-cell lymphomas, pancreatic, lung, breast & colon cancers
- Encoded by KSHV and Mareks disease viruses
- Activated by ALV, *rel* oncogene and EBV
- 6/6 Bic transgenic mice die of B-cell lymphomas in 6 months (Croce 2006)
- miR-155 is upregulated by inflammatory response in macrophages (Baltimore 2007)
- Bic knock-out mice defective in immune function (Bradley 2007); Bic needed to regulate germinal center response (Rajewsky 2007)

  Bic targets DNA repair machinery (Croce 2010)

# miR-155 is over-expressed in B-Cell lymphomas (human and chicken)




Splinted ligation assay

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# Avian Retroviruses

- Avian leukosis virus


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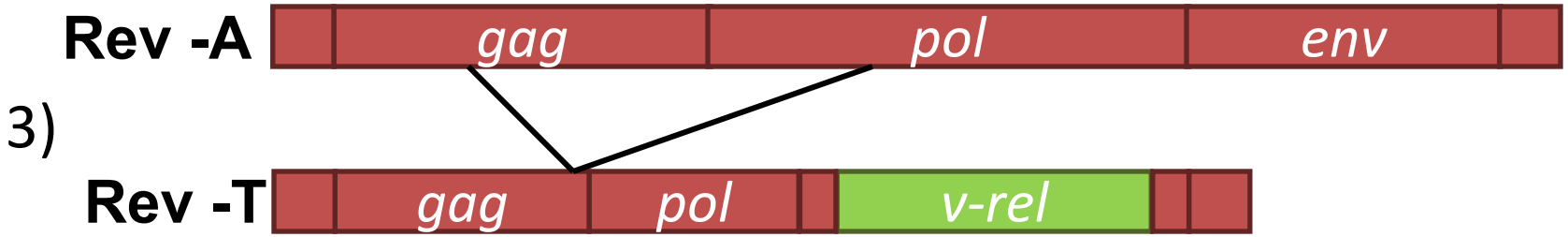
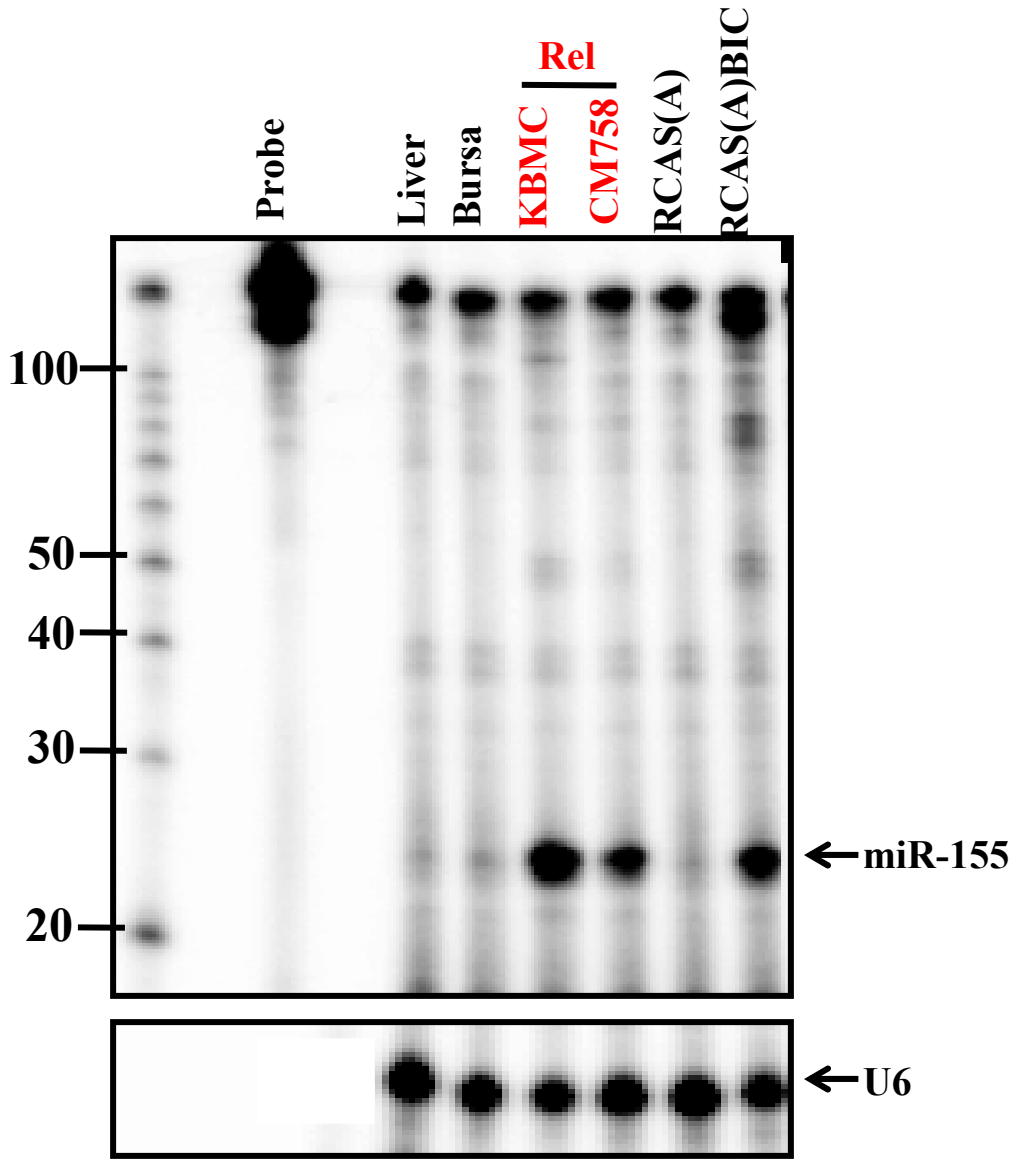
- Reticuloendotheliosis virus strain T (Replication deficient)

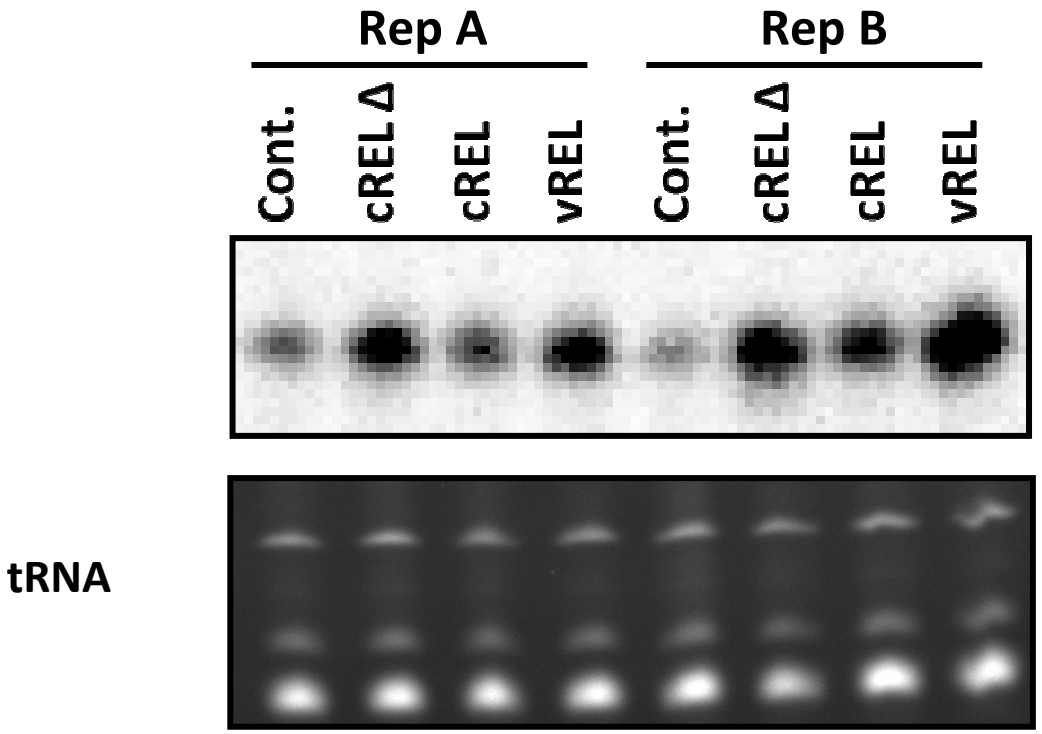
3)



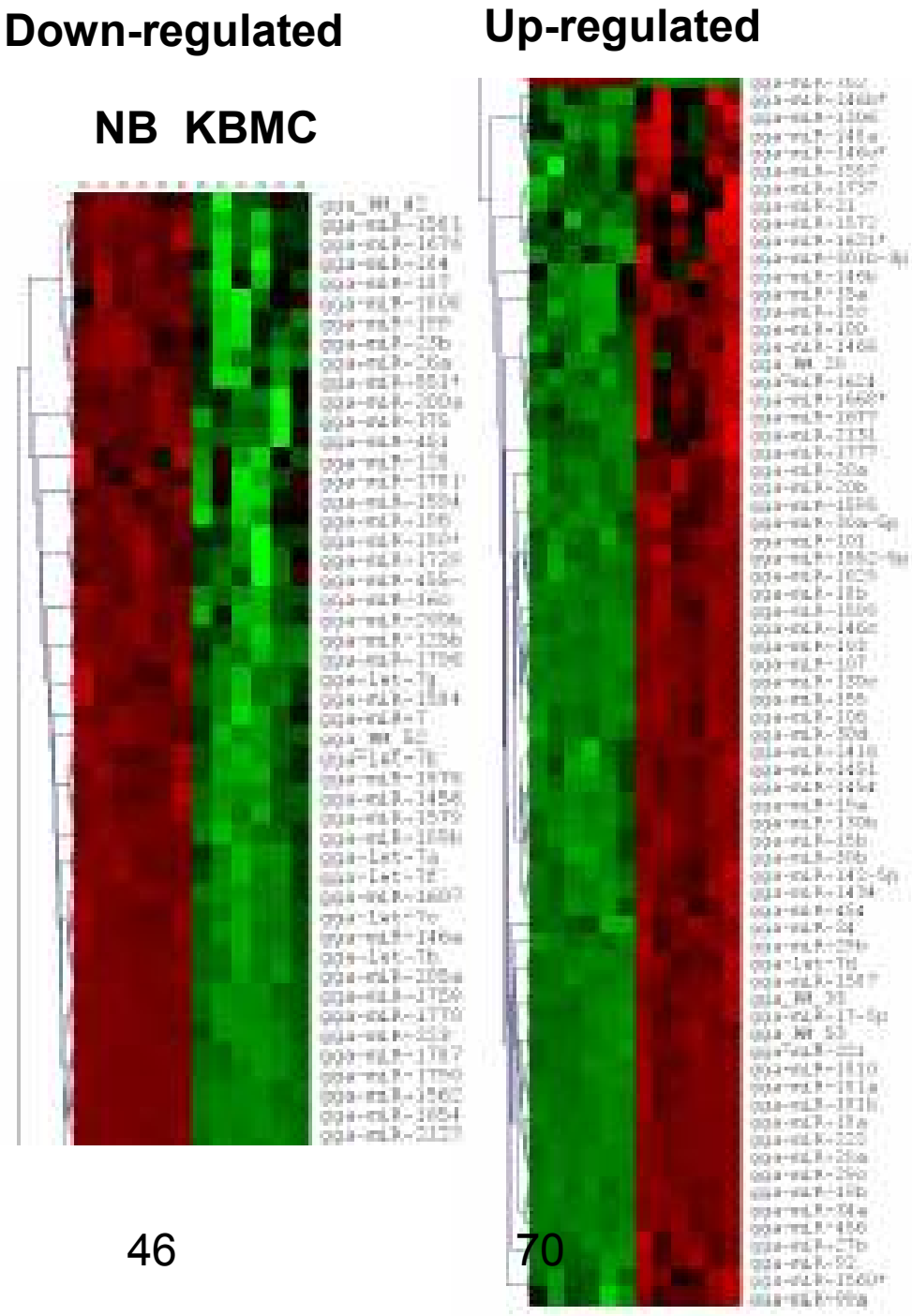
# REV-T derived B-cell lymphomas have increased miR-155 levels



# v-Rel upregulates miR-155 in CEFs



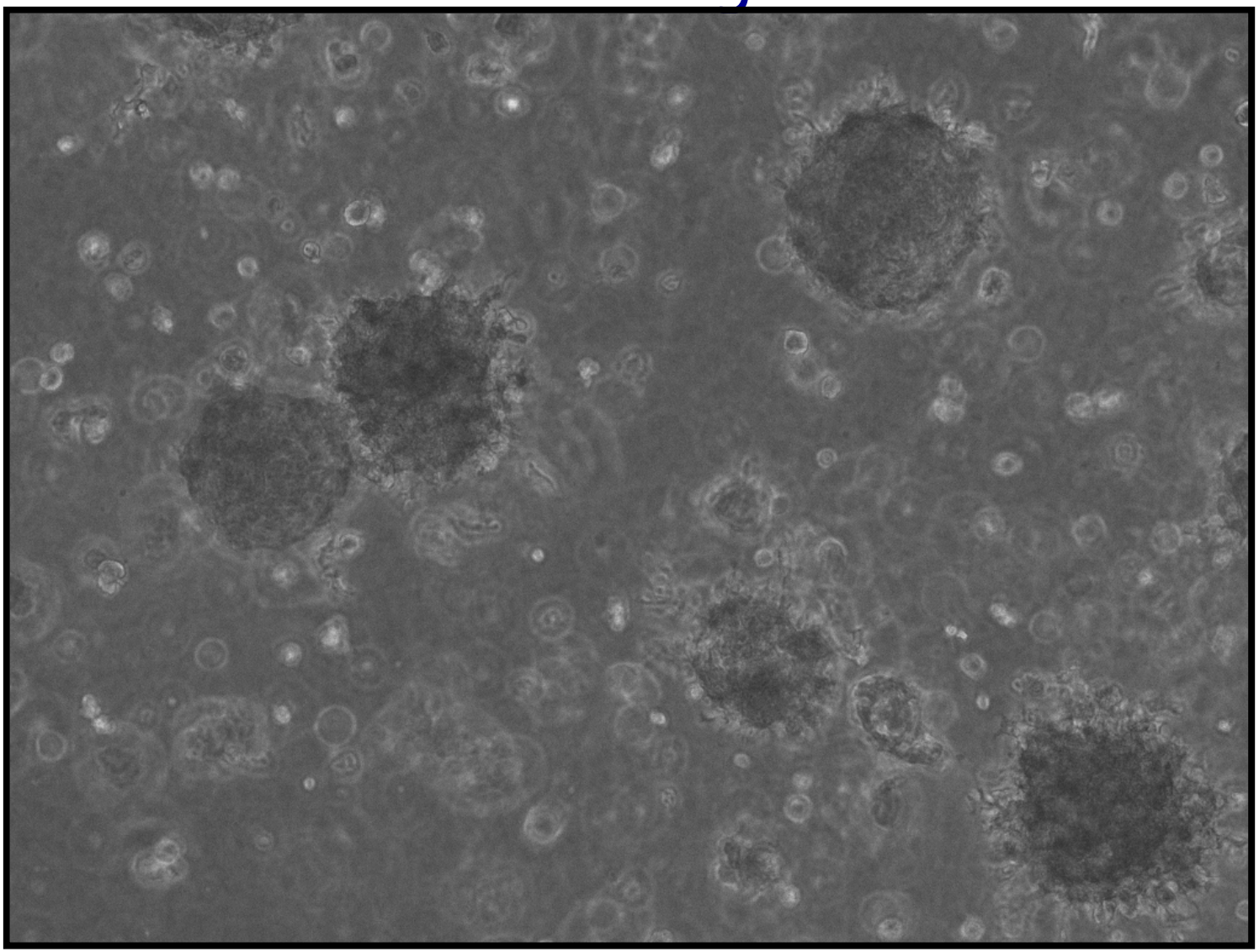
# Many miRNAs up in v-*rel* B-cell line




- Most previously identified oncogenic/tumor suppressive miRNAs are correspondingly regulated
  - miR 17-92 cluster is upregulated (4 – 9 fold)
  - miR-18 family is upregulated (10 – 20 fold)
  - **miR-155 is upregulated (40 fold) – highest expressed miRNA**
  - let-7 family miRNAs are downregulated (4 – 25 fold)
  - miR-34b is dowregulated (8 fold)

- However two known tumor suppressors are upregulated:
  - miR-29 family is upregulated (>32 fold)
  - miR-34a is upregulated (32 fold)

# v-Rel transformed B/T cells form colonies in soft agar



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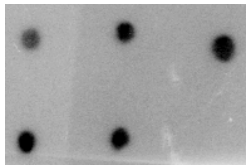
# miR-200a+b inhibit colony formation

Infect 160/12 cells with corresponding miRNA

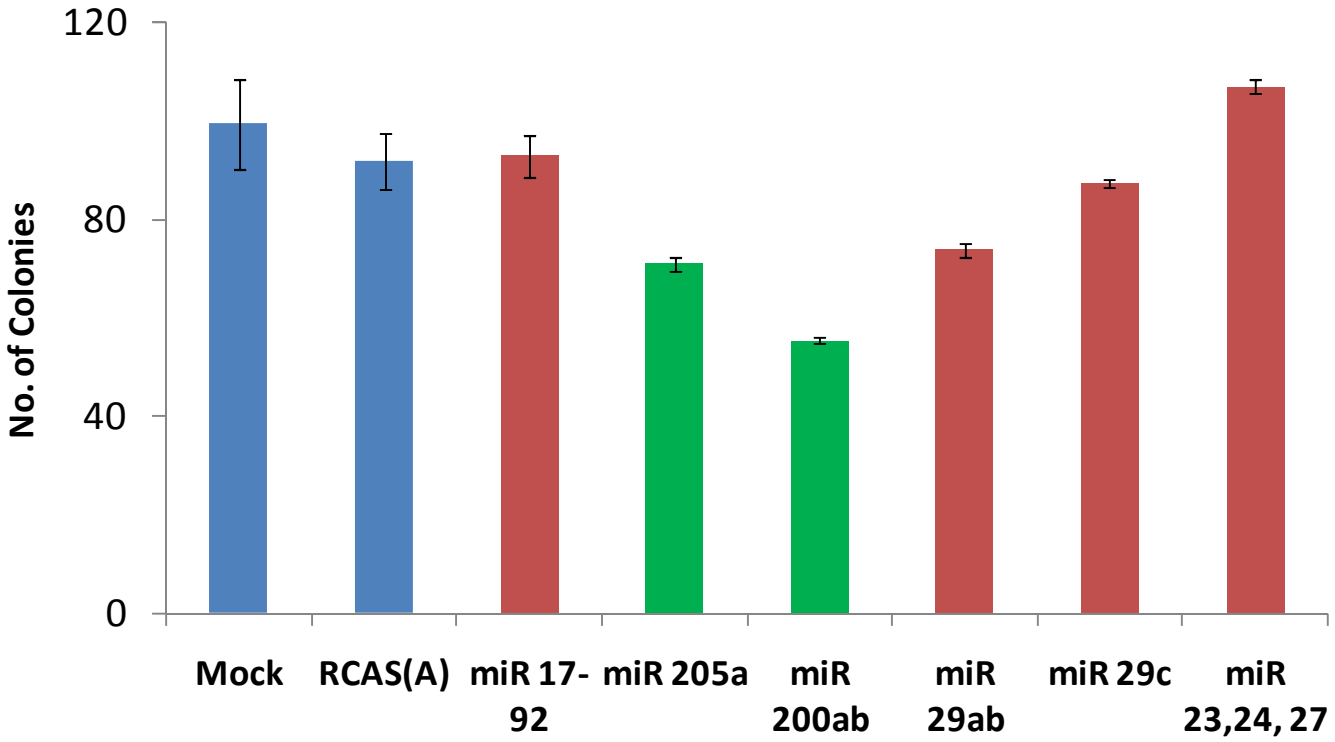
Check for infection

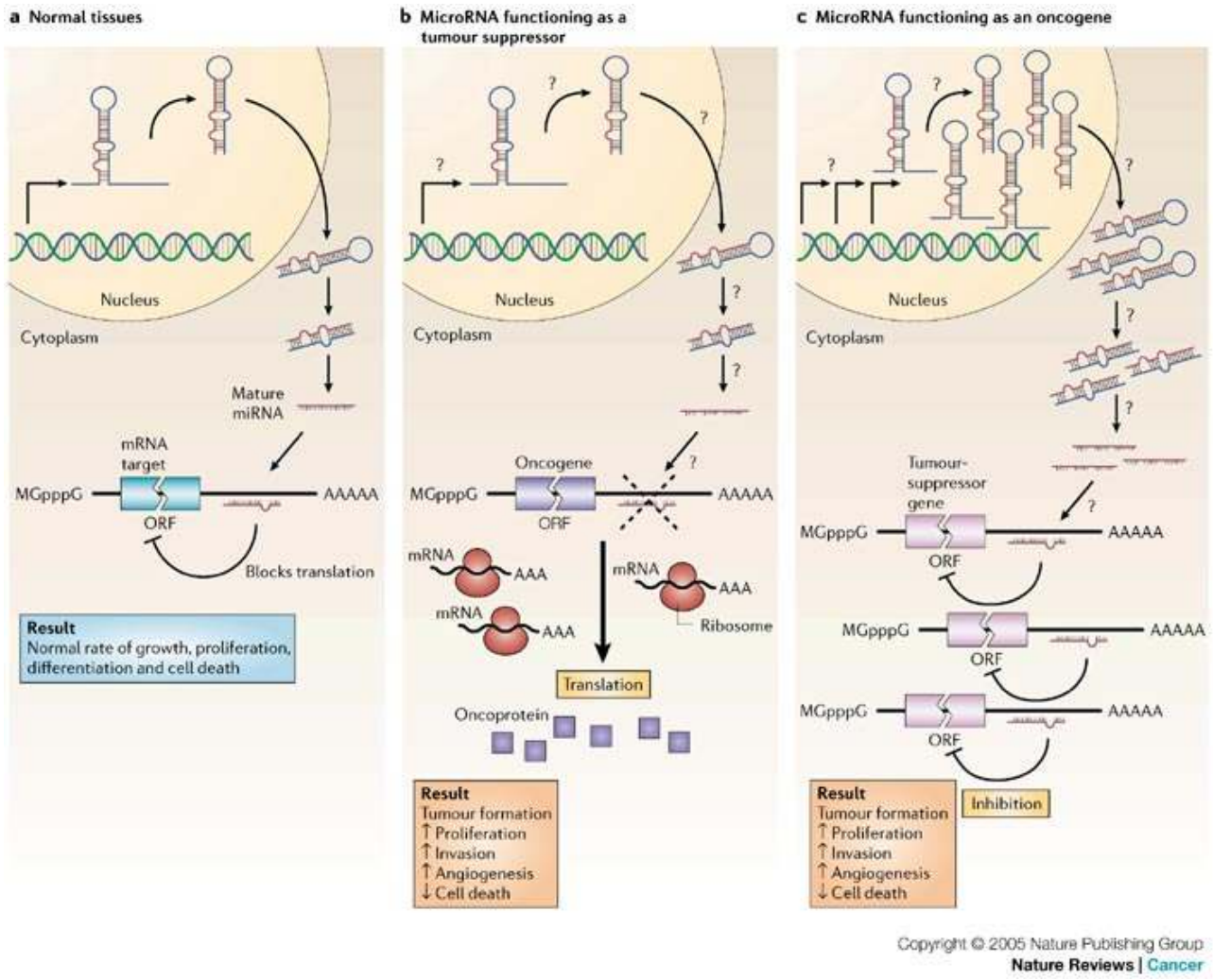


Check for miRNA expression

Plate 6 x $10^6$ cells in soft agar

Count colonies/quadrant 14 days



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Nature Reviews | Cancer

Esquela-Kerscher *et al. Nature Reviews Cancer* **6**, 259–269 (April 2006) | doi:10.1038/nrc1840

nature REVIEWS CANCER

# Conclusions

- v-Rel deregulates many different miRNAs in B-cell lymphomas, including miR-155. More miRs are induced than repressed.

- MAPK signaling pathways regulate subset of these miRNAs through AP-1 transcription factors

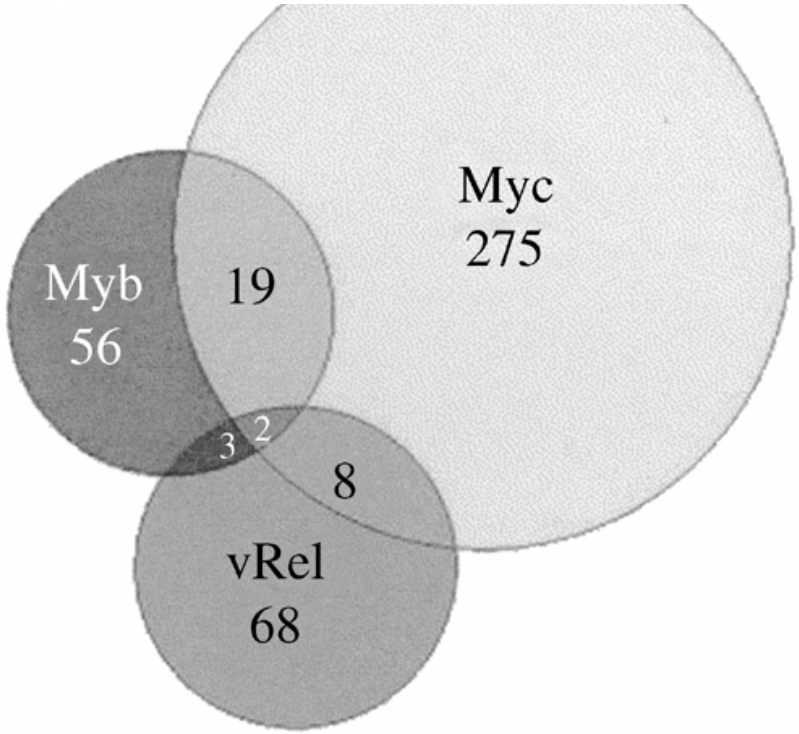
- miRNAs repressed by v-Rel (miR 200ab) diminish v-Rel transformation capability

# miRNA regulation may be a more general indicator of lymphomas than proteins

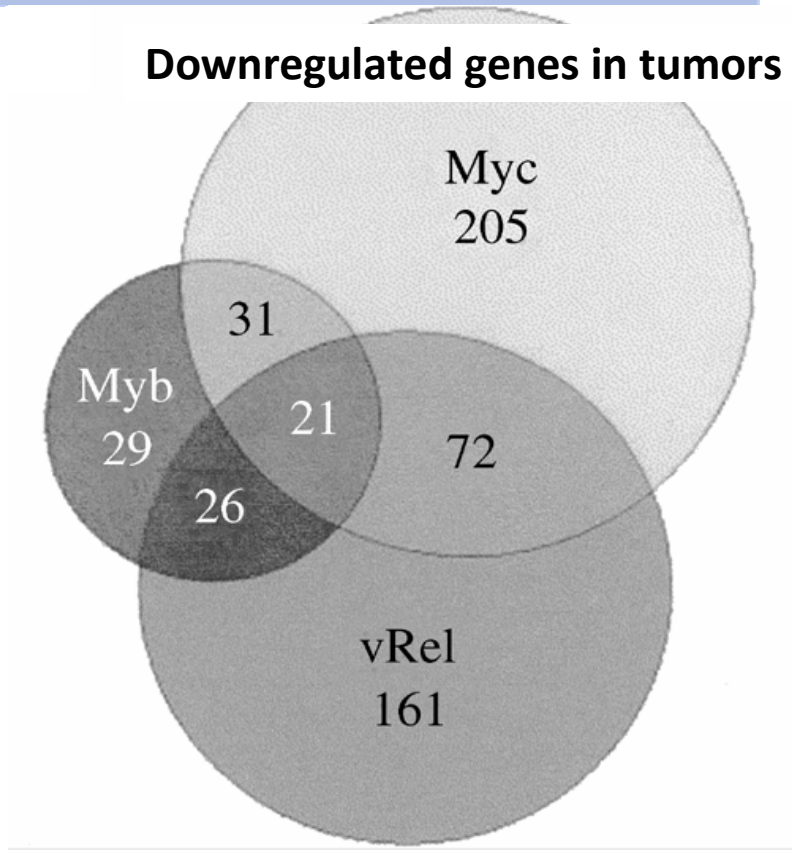
**Functional genomic analysis reveals distinct neoplastic phenotypes associated with c-*myb* mutation in the bursa of Fabricius**

Paul E Neiman[1,2], Jovana J Grbiç[1], Tatjana S Polony[3], Robert Kimmel[1], Sandra J Bowers[1], Jeffrey Delrow[1] and Karen L Beemon[3]

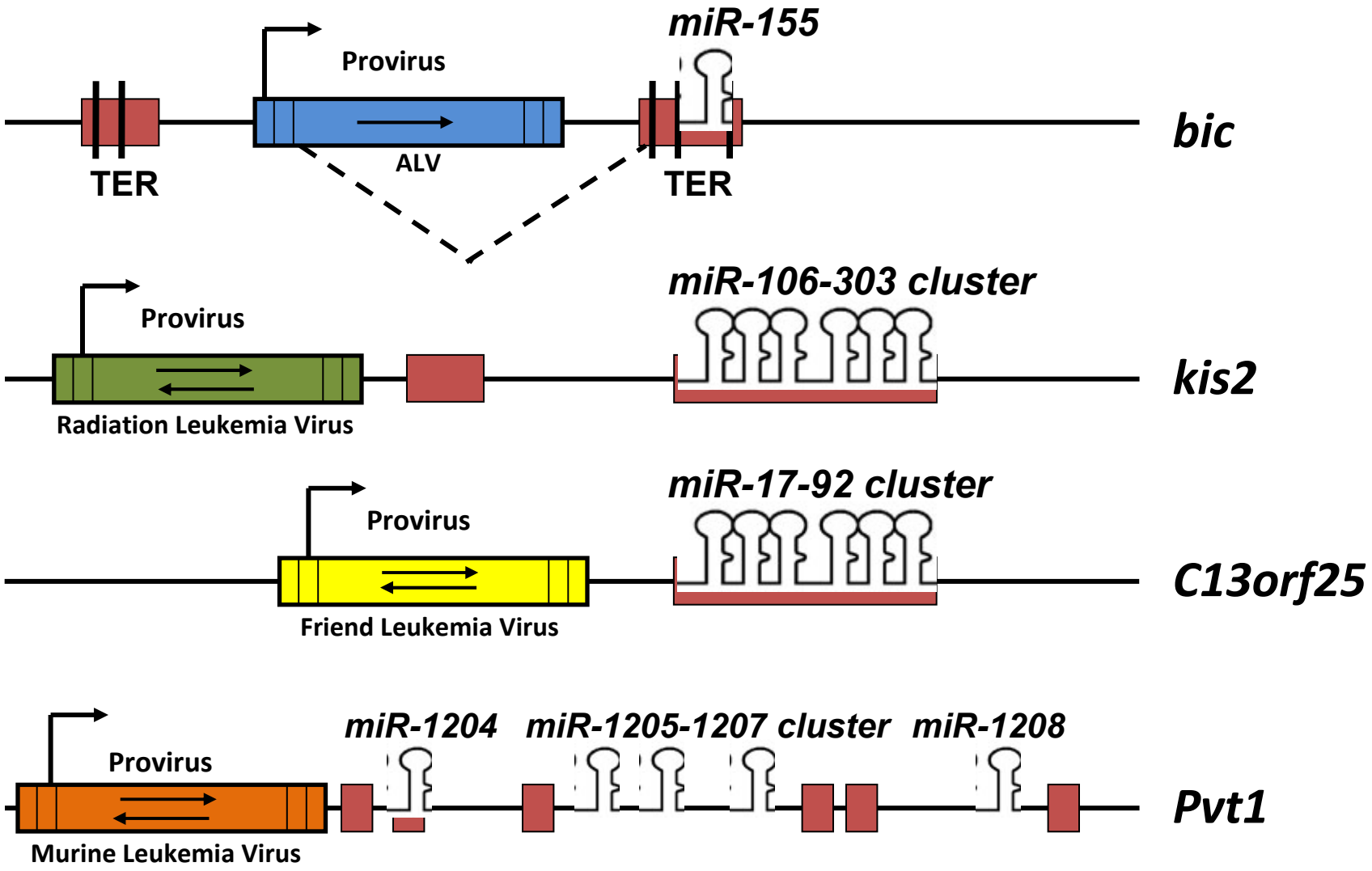
**Upregulated genes in tumors**




**Downregulated genes in tumors**

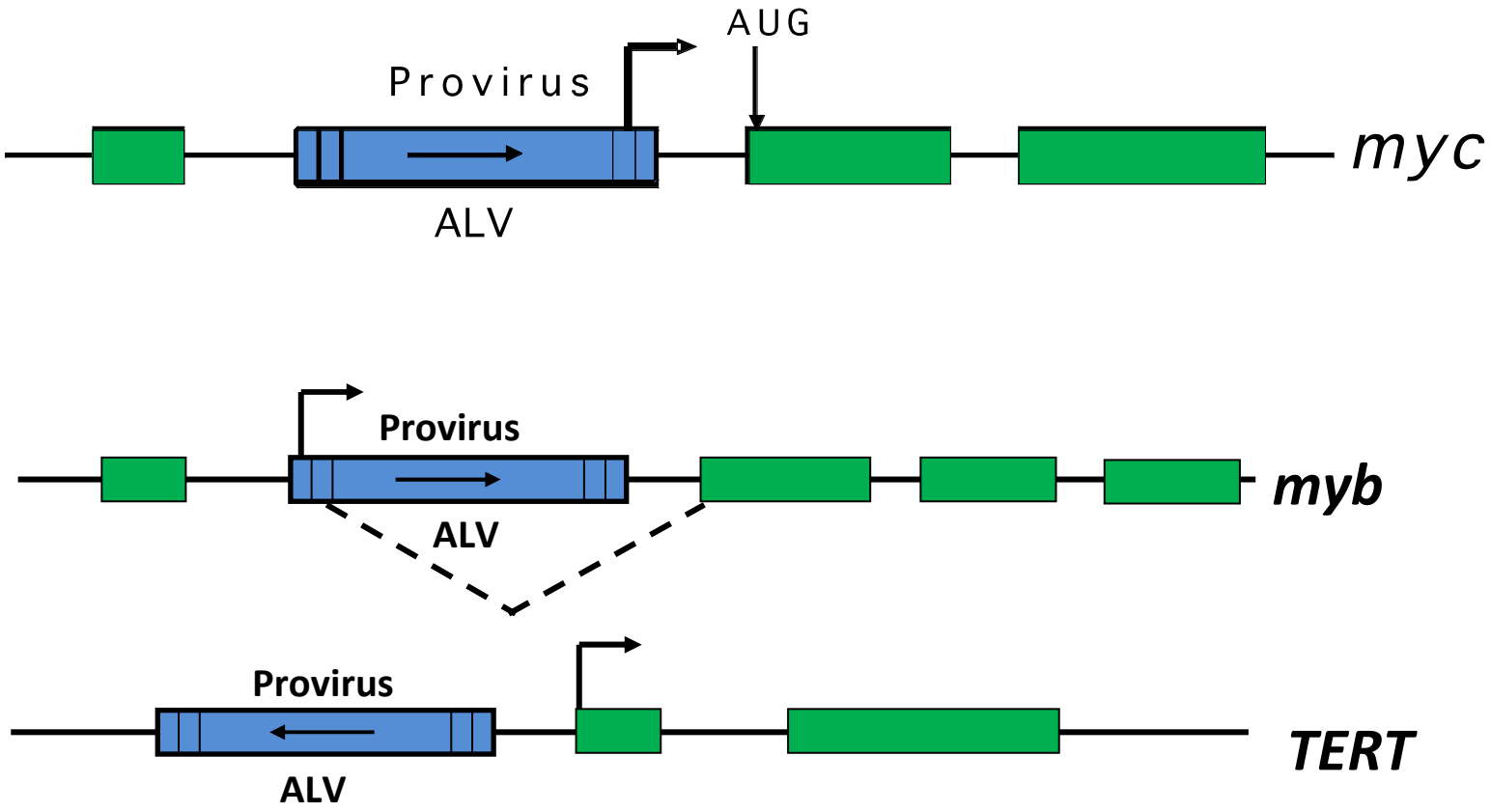


# Retroviruses identify oncogenic miRNAs



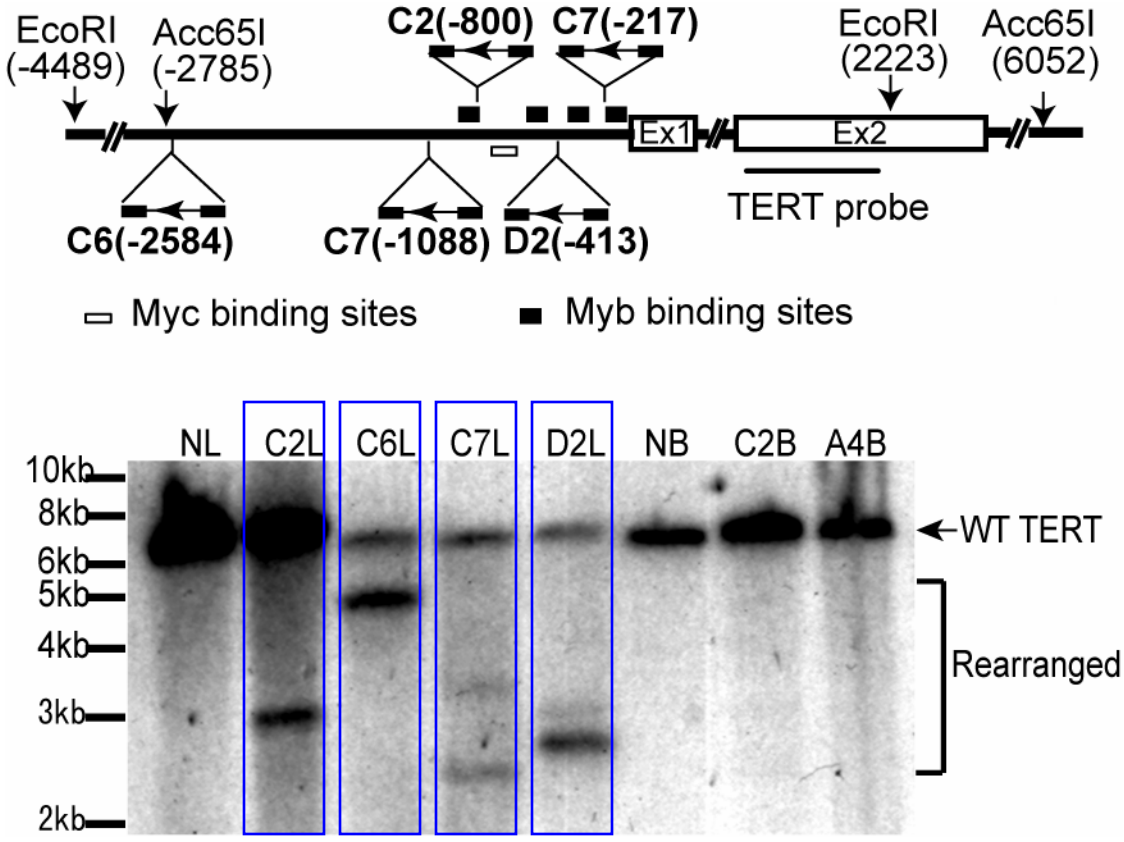

Clurman and Hayward 1989; Landais et al. 2005, 2007; Cui et al. 2

# ALVs induce tumors by insertional mutagenesis




Kanter et al. 1988; Pizer and Humphries 1989; Yang et al. 2007; Hayward et al. 1981

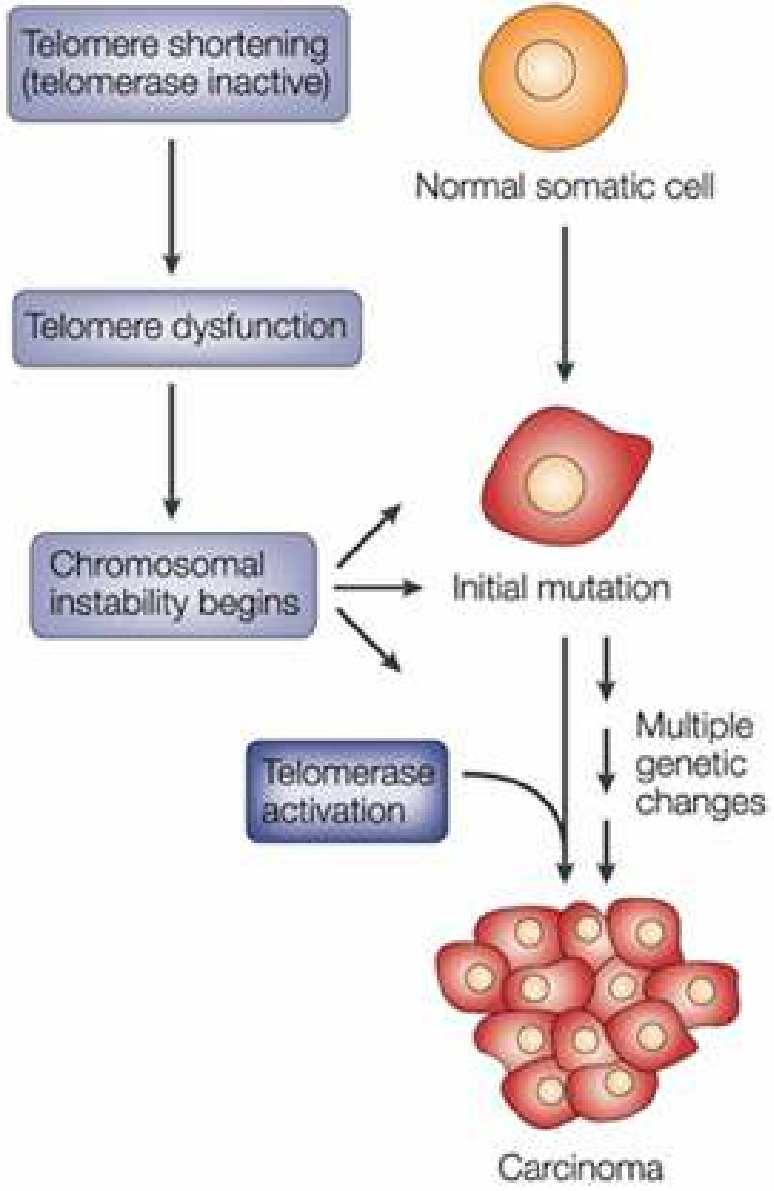
# Common ALV integrations upstream of TERT in B cell lymphomas




Southern blotting

Yang et al.
PNAS 2007

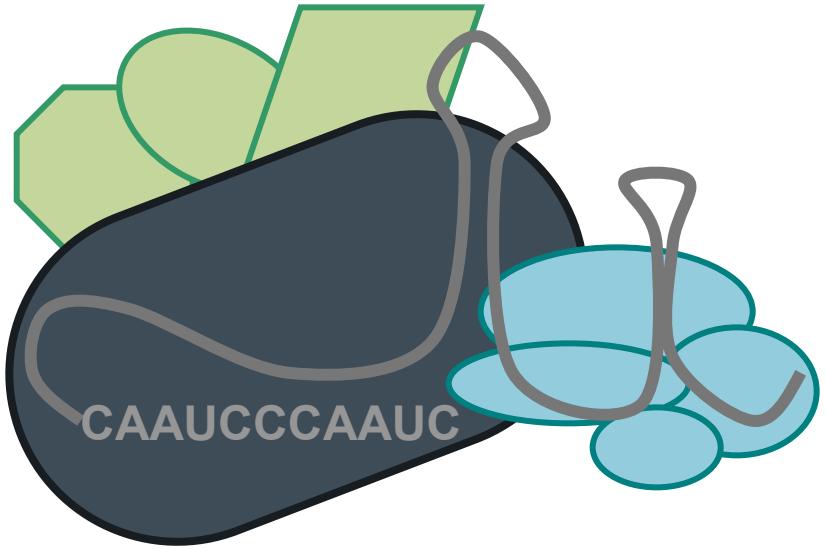
# What is the role of Telomerase in cancer?




Greider 2003

Nature Reviews | Cancer

# Human telomerase




**hTERT: telomerase protein**

**hTR: telomerase RNA**

## Canonical Functions

- Protect telomere repeats from erosion
- Prevent chromosome fusions
- Immortalize cells

## Non Canonical Functions

hTR

- Response to DNA damage
- Promotes cell growth
- Mitochondrial localization
- Reduces neurotoxicity
- Inhibits apoptosis

# SUMMARY

OncomiR-155: promotes cell survival
Targets JARID2
Upregulated by Rel transcription factor and
ALV integration

TERT appears to be activated in B cell tumors

Are other types of non-coding RNAs activated by ALV integration in tumors?

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